Sulfonated Perfluorochemicals in the Environment: Sources, Dispersion, Fate and Effects

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1.0 Preface

This paper provides an overview of 3M's current knowledge about the sources, dispersion, fate and effects of some of its fluorinated chemical products. It specifically addresses sulfonated perfluoronated chemistry and products, with the major focus on those compounds with an eight carbon chain structure. There are other fluorinated chemical products but these are not covered in this white paper.

The paper presents the past testing of these chemicals for environmentally relevant properties and assesses the quality and adequacy of past testing. It also presents recent results of environmental sampling, estimates of quantities of wastes generated at manufacturing plants and from product use, and new data on physical, chemical and ecotoxicological properties of sulfonated perfluorochemicals. It describes in detail the comprehensive exposure assessment plan currently being implemented. This plan is aimed at providing a better understanding of the transport, fate and effects of these chemicals in the environment and will help the company determine appropriate future actions.

As these studies return data, test plans will be revised to incorporate new information. For this reason, the results of present testing should be treated cautiously. Some data represent first attempts at characterization of complex chemicals in very difficult and dynamic environmental test matrices. The program incorporates new analytical technology, complex models and many variables. These initial findings are subject to change as results from currently planned testing on degradation, biological receptors, wastes from manufacturing facilities and other exposure data are obtained.

This paper should be read in conjunction with previous submittals about the health and environmental issues associated with 3M's sulfonated perfluorochemical product line. In January 1999, 3M submitted to the Environmental Protection Agency (EPA) a report, Perfluorooctane Sulfonate: Current Summary of Human Sera, Health and Toxicology Data, that provided details of analyses of pooled blood sera samples that demonstrated the presence of perfluorooctane sulfonate (PFOS) at very low levels. In February 1999, 3M provided a comprehensive review, The Science of Organic Fluorochemistry, describing the health effects and background chemistry associated with PFOS. Another report submitted to EPA in May 1999, Fluorochemical Use, Distribution, and Release Overview, describes how 3M produces sulfonated perfluorochemicals, which product lines incorporate them, and the uses for these products. Finally, various Section 8(e) submissions have been forwarded to EPA relative to these sulfonated perfluorochemicals.

2.0 Executive Summary

3M produces sulfonated perfluorochemicals by an electrochemical fluorination process. This process creates a complex and variable mix of chemicals in which fluorine atoms replace hydrogen atoms on the organic feedstock and carbon-carbon bonds are rearranged. Because of the carbon-fluorine bond formed by this process, the compounds created are considered to be very stable. Perfluorochemicals have complete substitution of fluorine for hydrogen. Fluorochemicals can repel both water and oils, reduce surface tension dramatically, act as catalysts for oligomerization and polymerization, and function under extreme conditions. Major uses for sulfonated perfluorochemicals are surface protectors and surfactants.

Fluorine's high electronegativity confers a strong polarity to carbon-fluorine bonds, contributing to the stability and nonreactive character of perfluorochemical molecules. They are unusual as that perfluoroalkyl chains are both oleophobic and hydrophobic. The addition of charged moieties to the chain may affect the water solubility of the shorter chains.

The highest volume sulfonated perfluorochemical produced by 3M is perfluorooctanesulfonyl fluoride (POSF). After synthesis, it is used to create several product lines. During their life cycles, POSF and POSF-based products may degrade. If degradation occurs, current research suggests perfluorooctane sulfonate, (PFOS) and a few other perfluorinated forms are degradation products. Timeframes for degradation are variable, with some polymeric products apparently stable for very long periods of time.

The identification and quantification of sulfonated perfluorochemicals pose difficult analytical challenges. Reliable methods for extraction, separation and identification of sulfonated perfluorochemicals in tissues and environmental matrices have evolved and have been developed only in the last few years. New analytical technology is providing capabilities of detection in wide varieties of matrices at parts per trillion (ppt) levels and identification of metabolites and breakdown products.

As fully described throughout this paper, completion of a comprehensive exposure assessment and related scientific studies will require many years of intensive research. 3M is pursuing an aggressive program to reduce releases to the environment while that scientific research is being conducted. It is not the purpose of this paper to describe the nature and extent of that undertaking. Readers should be made aware, however, that 3M has initiated a wide range of activities to utilize available opportunities for reductions in releases. These have included installation of new controls to reduce waste streams in 3M manufacturing facilities. They have also included product stewardship efforts to communicate to customers and downstream users, information regarding fluorochemicals and the need to exert careful management over these substances. In addition, 3M has undertaken major efforts to reinvent its products through the use of alternative chemistry to reduce the volume of fluorochemicals used in those products. All of these efforts will

be continued with intensity while the scientific research described in this paper is being carried out.

3M is examining the life cycle of its sulfonated perfluorochemical products to identify releases to the environment from manufacturing processes, supply chains, product use and disposal. First it is determining waste streams generated throughout the life cycle. This information will be used to estimate environmental releases. This approach is necessary since not all waste produced is released to the environment. Manufacturing waste studies are underway at the 3M plant in Decatur, Alabama on POSF-based processes. PFOS-based waste streams generated from these manufacturing processes are conservatively estimated to be about 1.1 million pounds per year, about 90% as solid waste, most of which is incinerated and destroyed. Recent wastewater controls have reduced amounts of PFOS actually discharged to the river by half since 1998.

Data from business units have identified key products that contain the majority of the fluorochemical solids sold in the United States in 1997. Using this sales information, 3M estimated customer and end user waste streams. Most of the waste generated from these sources is in the form of solid waste. Releases to the environment from product disposal to landfills, wastewater treatment plants and incineration are all being investigated.

Several different fate and transport mechanisms have been identified as important to study. Initially models are being used for screening-level assessments of potential fate mechanisms. Multi-media fugacity models are under development to incorporate the unique properties of fluorochemicals.

Sulfonated perfluorochemicals have been detected at low levels in some species of eagles and wild birds. Low levels were detected in bird plasma and bird livers. 3M believes that these sets of data are insufficient to draw conclusions with any statistical merit. In screening sampling of the river and sediments near the Decatur manufacturing plant, PFOS was present in a few samples collected near the outfall. All this information was used in the design of a more comprehensive program of biosphere sampling. The goal of the biosphere sampling plan is to screen for PFOS across a range of species, habitats and geographic locations and to identify areas on which to focus scientific investigation to develop a better understanding of any potential environmental effects.

A multi-cities study will determine environmental distribution and potential sources of human and ecological exposure. The multi-cities study pairs cities with significant manufacturing or commercial use of fluorochemical products with cities of the same size without significant use. Levels of PFOS and its precursors will be measured in food, air, water, sediment, and disposal facilities. Additionally, levels are being measured arising from carpet use, product uses and potential migration into food from packaging.

The role of hydrolysis, photolysis and biological processes in the degradation of sulfonated perfluorochemicals is being studied. Research suggests that the biodegradation of fluorinated sulfonates requires the presence of hydrogen at the alpha

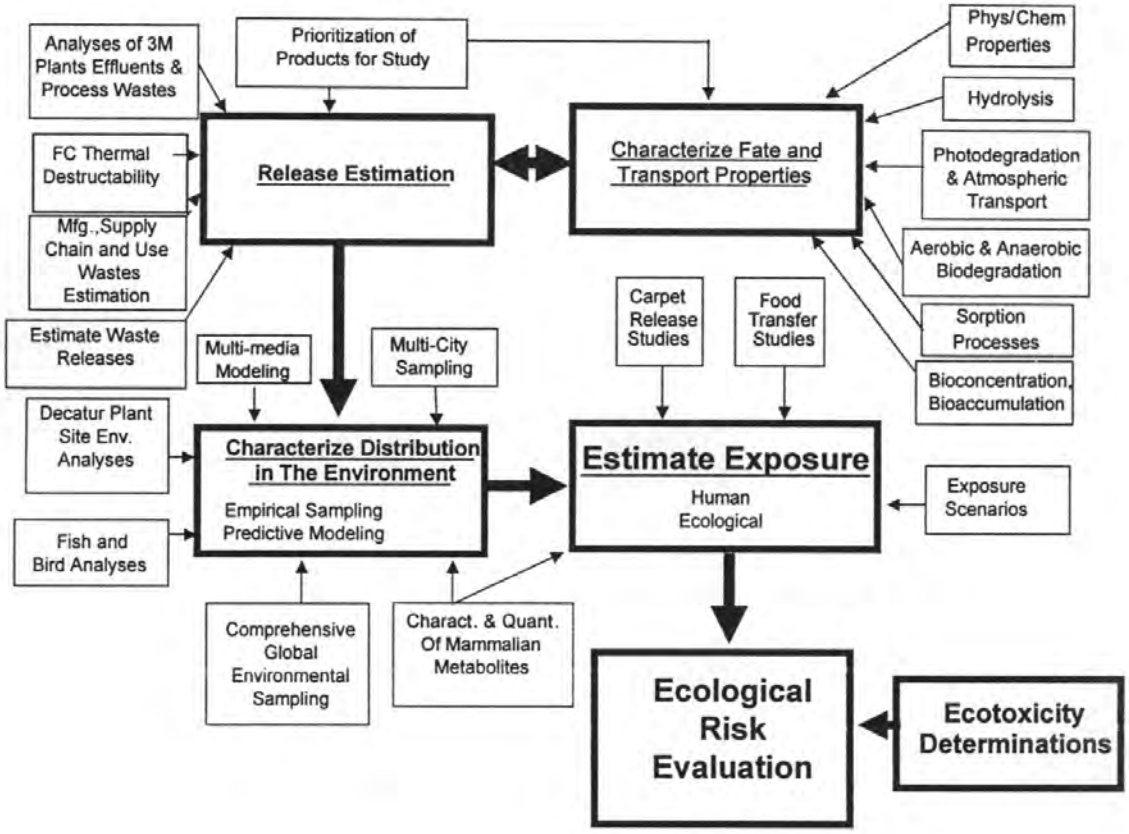
carbon on the fluorinated chain and that perfluorinated molecules are susceptible to breakdown only at non-fluorinated side chains. Degradation of sulfonated perfluorochemicals is not complete but results in production of other fluorochemicals. Studies suggest that compounds made from POSF, a commercially important perfluorochemical product and intermediate, are transformed during metabolism to another sulfonated perfluorochemical, PFOS. PFOS does not appear to further degrade except by incineration.

Several sulfonated perfluorochemicals have been subjected to basic screening tests for environmental toxicity. Different species varied significantly in their response to the same chemical even when using the same laboratory procedure. New testing is underway using purified sulfonated perfluorochemicals, measured test concentrations, and a wide variety of test organisms. Results of these studies are reported in this white paper.

The research projects that are yielding new information on sulfonated perfluorochemicals are part of a comprehensive plan to assess the potential pathways of environmental exposure associated with the manufacture, use and disposal of sulfonated perfluorochemical products. Figure 1 portrays the plan components. Work on the plan is now underway using a combination of 3M resources and outside experts. Recent analytical advances and this extensive research effort are expected to contribute significantly to a better understanding of environmental fate and effects.

The findings resulting from the comprehensive plan, along with new ecotoxicological test data, will be used to evaluate ecological risk. While this evaluation is underway, 3M is implementing actions to reduce generation of waste in manufacturing processes and to reduce releases of sulfonated perfluorochemicals into the environment through process improvements, waste reduction and engineering redesign.

Figure 1. Diagram of Fluorochemical Assessment Plan.



3.0 Introduction to Fluorochemicals

Fluorochemicals are components of several important 3M product lines due to their unique and useful properties. They are stable, chemically inert and generally nonreactive. As components of products, they repel both water and oil, reduce surface tension much lower than other surfactants, act as catalysts for oligomerization and polymerization, and function where other compounds would rapidly degrade.

3M has produced fluorochemicals commercially for over 40 years. 3M produces fluorochemicals by combining anhydrous hydrogen fluoride with hydrocarbon stock in the presence of electrical energy. The highest volume sulfonated fluorochemical produced by 3M is perfluorooctanesulfonyl fluoride (POSF).

$$C_8H_{17}SO_2F + 17\,HF \xrightarrow{4.5\text{-}7.0\ V} C_8F_{17}SO_2F + 17\,H_2$$

1-Octanesulfonyl fluoride — Perfluorooctanesulfonyl fluoride **(POSF)**

The fluorination process overall yields about 35-40% straight chain (normal) POSF, and a mixture of byproducts and waste of uncharacterized and variable composition containing:

-higher or lower straight chain homologues, n-$C_nF_{2n+1}SO_2F$, of various chain lengths (7% of process output)

e.g. $C_6F_{13}SO_2F$, $C_7F_{15}SO_2F$, $C_9F_{19}SO_2F$

-branched chain perfluoroalkyl products of various chain lengths (18-20% of output)

e.g. $CF_3CF_2CF_2CF_2CF_2CF(CF_3)CF_2SO_2F$ $\quad$ $CF_3CF(CF_3)CF_2CF_2CF(CF_3)CF_2SO_2F$

- straight chain, branched and cyclic perfluoroalkanes and ethers (20-25% of output) e.g. CF_4 C_2F_6, C_3F_8, C_4F_{10}, C_5F_{12}, cC_4F_8

-"tars" (high molecular weight fluorochemical byproducts) and other byproducts, including molecular hydrogen (10-15% of output).

Because of slight differences in process conditions, raw materials, and equipment, the mixture produced by the electrochemical fluorination process varies somewhat from lot to lot and from plant to plant. Numerous process steps are used to convert the fluorinated mixture into final products.

The largest production of fluorochemicals occurs at the 3M manufacturing plant in Decatur, Alabama, and this plant is the focus of current studies. During production, many byproducts and waste products are formed. The volatile waste products have been vented to the atmosphere in the past but improvements are underway to capture and destroy these releases by thermal oxidation. The tars are disposed at hazardous waste landfills or treated by incineration. The byproducts, many of which are incompletely fluorinated with hydrogen atoms still present, are recycled back into processes or partially degraded in stabilization processes and discharged to wastewater treatment systems. The treatment sludge is landfilled. Some of the non-POSF-based byproducts are recovered and sold for secondary uses.

The product of the electrochemical fluorination process is thus not a pure chemical but rather a mixture of isomers and homologues. Perfluorochemicals have complete substitution of fluorine for hydrogen. The commercialized POSF derived products are a mixture of approximately 70% linear POSF derivatives and 30% branched POSF derived impurities. POSF is used as a product and is also an important intermediate in the synthesis of substances used in many other 3M products. To a lesser extent, homologues of POSF, [$C_nF_{(2n+1)}SO_2F$ where n= 2-9, exclusive of 8], are also components used in the formation of other 3M products.

Some of the POSF derived products are surface active materials and monomers of relatively low molecular weight (~500 daltons). These monomers are used as low molecular weight surfactants or are joined with other monomers to form higher molecular weight oligomers and polymers with a mix of fluorinated and unfluorinated portions. Fluorochemical monomers can also be joined to phosphates, to polymeric and oligomeric urethane, or to acrylate backbones through ester and other linkages. The majority of 3M's sulfonated perfluorochemicals produced are used in polymeric form for treatment of surfaces and materials. For example, fluorochemical containing polymers (urethanes, acrylics and esters) can provide soil, stain, and water resistance to personal apparel and home furnishings.

Some products synthesized from POSF and its homologues are sold as raw materials to customers who use them as intermediates or components of their products. The intermediates can be covalently bound to a variety of polymeric hydrocarbon backbones.

The 3M product lines that use sulfonated perfluorochemicals are summarized below. (Product lines using fluorochemicals that contain no sulfonyl groups are not listed.)

Surface Treatments

Fabric/Upholstery Protector (High molecular weight (MW) polymers)
Carpet Protector (High MW polymers)
Leather Protector (High MW polymers)
Paper and Packaging Protector (High MW phosphate esters or high MW polymers)

Surfactants (Low MW chemical substances)

Specialty surfactants
Household additives
Electroplating and etching bath surfactants
Coating and coating additives
Chemical intermediates
Carpet spot cleaners
Fire Extinguishing Foam Concentrates
Mining and Oil Surfactants

Other Uses

Insecticide Raw Materials (Low MW chemical substances)

Typically a fluorochemical product contains a small amount of fluorochemical residuals: unreacted or partially reacted starting materials or intermediates. Residuals which are common to formulations of sulfonated perfluorochemical products include: perfluorooctane sulfonate (PFOS), N-ethyl (or N-methyl) perfluorooctane sulfonamide (N-EtFOSA or N-MeFOSA) , N-ethyl (or N-methyl) perfluorooctane sulfonamidoethyl alcohol (N-Et FOSE alcohol or N-MeFOSE alcohol) and perfluorooctanoic acid (PFOA). Table 1 identifies some sulfonated perfluorochemicals, their acronyms, chemical name, and formulas.

Table 1. Perfluorochemical Glossary

Designation	Name	Formula
POSF	perfluorooctanesulfonyl fluoride	$C_8F_{17}SO_2F$
PFOS	perfluorooctane sulfonate	$C_8F_{17}SO_3^-$
PFOSH	perfluorooctanesulfonic acid	$C_8F_{17}SO_3H$
PFOS.NH_4 salt	ammonium perfluorooctanesufonate	$C_8F_{17}SO_3NH_4$
PFOS.DEA salt	Perfluorooctanesulfonate diethanolamine salt	$C_8F_{17}SO_3NH(CH_2CH_2OH)_2$
PFOS.K salt	potassium perfluorooctanesulfonate	$C_8F_{17}SO_3K$
PFOS.Li salt	lithium perfluorooctanesulfonate	$C_8F_{17}SO_3Li$
FOSA	perfluorooctanesulfonamide	$C_8F_{17}SO_2NH_2$
PFOSAA	perfluorooctane sulfonylamido (ethyl)acetate	$C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$
PFDS	perfluorodecanesulfonate	$C_{10}F_{13}SO_3^-$
PFHS	perfluorohexane sulfonate	$C_6F_{13}SO_3^-$
N-EtFOSA	N-ethyl perfluorooctanesulfonamide	$C_8F_{17}SO_2NHC_2H_5$
N-MeFOSA	N-methyl perfluorooctanesulfonamide	$C_8F_{17}SO_2NHCH_3$
N-EtFOSE alcohol	N-ethylperfluorooctane sulfonamidoethanol	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
N-MeFOSE alcohol	N-methylperfluorooctane sulfonamidoethanol	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$
N-EtFOSEA	N-ethylperfluorooctanesulfonamidoethyl acrylate	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OCOCH=CH_2$
N-EtFOSEMA	N-ethylperfluorooctanesulfonamidoethyl methacrylate	$C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OCOC(CH_3)=CH_2$
N-MeFOSEA	N-methyl-perfluorooctanesulfonamidoethyl acrylate	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OCOCH=CH_2$
PFOA	perfluorooctanoic acid	$C_7F_{15}CO_2H$

4.0 Physical-Chemical Properties of Fluorochemicals

Fluorinated organics are less well described in the science literature than organic molecules bearing other halogens, i.e. bromine and chlorine, which have been more thoroughly investigated by many researchers in published reports. To understand the properties of fluorinated organics, it is necessary to describe the properties of fluorine. Fluorine has several characteristics that differ from the other halogens and contribute to the unusual properties of fluorochemicals.

Fluorine has a van der Waals radius of 1.35 Å, more comparable to that of oxygen than other halogens, and isosterically similar to a hydroxyl group. Fluorine has the highest electronegativity (4.0 -Pauling scale) of all the halogens, indeed the highest in the periodic table. This confers a strong polarity to the carbon-fluorine bond. The carbon-fluorine bond is one of the strongest in nature (~110 kcal/mol). This very strong, high energy bond contributes to the stability of fluorochemicals.

The high ionization potential of fluorine (401.8 kcal/mole) and its low polarizability leads to weak inter- and intramolecular interactions. This is demonstrated by the low boiling points of fluorochemicals relative to molecular weight, and their extremely low surface tension and low refractive index. The partitioning behavior of perfluoroalkanes is unusual. Some perfluoroalkanes when mixed with hydrocarbons and water form three immiscible phases, demonstrating that perfluorinated chains are both oleophobic and hydrophobic. A charged moiety, such as carboxylic acid, sulfonic acid, phosphate or a quaternary ammonium group, when attached to the perfluorinated chain, makes the molecule more water soluble because of the hydrophilic nature of these charged moieties. Therefore, such functionalized fluorochemicals can have surfactant properties. Typically, the presence of these charged groups on short chain perfluorinated compounds (<C6) noticeably increases the solubility of the compound in water.

Physical data available on fluorochemicals at 3M have been principally those parameters needed for quality control use and material handling. Table 2 summarizes the physical data for low molecular weight, POSF-based fluorochemical products that have been developed for use on Material Safety Data Sheets (MSDS).

Some of these perfluorochemical products are primarily used as surfactants; others are primarily used as intermediates in the formation of polymeric or oligomeric products. Some of these low molecular weight fluorochemicals are also likely intermediates in the degradation of polymeric compounds. Some can also result from environmental transformation of other low molecular weight fluorochemical products. It is important to remember that these data were obtained using products that were not highly refined, and products may have more than one fluorochemical component. Some may have nonfluorochemical components that enter into determination of the values. Because of improvements in analytical techniques and product refinement, these data are in the process of being replaced by better quality data.

Table 2. Physical Data on Fluorochemical Products (Developed for Use on MSDS Sheets)

Source: MSDS Sheets
Abbreviations: N/D: not determined; N/A: not applicable; ~: approximately
*measured at 1mm Hg #measured at 2 mm Hg

Product Use	Principal Fluorochemical	boiling pt (b) melting pt, (m) °C	vapor pressure mmHg calc. @20°C	vapor density calc. @20°C Air=1	evap rate BuOAc =1	solubility in water	Specific Grav. Water=1	pH
Intermed.	POSF	154 b	<10	>1.0	<1.0	neglig	~1.8	N/A
Intermed.	N-MeFOSE alcohol	75-95 m	N/D	N/D	N/D	neglig	~1.7	N/A
Intermed.	N-EtFOSE alcohol	~118 b*	<10	>1.0	<1.0	neglig	~1.7	N/A
Surfactant	N-EtFOSA	~110 b# ~ 90 m	<10	>1	N/D	neglig	~1.6	N/A
Intermed.	N-EtFOSEA	~150 b*	<10	>1.0	<1.0	nil	~1.5	N/A
Intermed.	N-EtFOSEMA	~150 b*	<10	>1.0	<1.0*	neglig	~1.5	N/A
Surfactant	PFOS NH_4^+ salt	~ 82 b	~34	~1.0	<1.0	moderate	~1.1	~7
Surfactant	PFOS Li salt	~100 b		N/D	<1	complete	~1.1	6-8
Surfactant	PFOS K salt	N/A	N/A	N/A	N/A	slight	~0.6	7-8
Surfactant	PFOS DEA salt	~ 98 b	~31	~0.62	<1.0	complete	~1.1	~7
Surfactant	PerfluoroC10 sulfonic acid, NH_4^+ salt	~ 96 b	~16	~1.08	<1	moderate	1.08	8.5-9.5
Surfactant	Glycine derivative of FOSA	~100 b	~18	~0.87	<1.0	complete	~1.3	~11
Surfactant	N-EtFOSE alcohol, ethylene oxide adduct	210 b	~18	0.64	<1	apprec	1.31-1.34	5.5-8.4

Additional physical data were developed in the mid-1970s and early 1980s on a few, high volume products. Typically these data are related to developing an understanding of environmental fate, e.g. data on soil mobility and partitioning coefficients. They are summarized in Table 3. 3M has evaluated these data for reliability and the reliability codes are included as part of the table. Progress in analytical techniques has significantly improved the reliability of current data compared to the reliability of these historical data. Current physical/chemical data are found in Table 4.

Table 3. Historical Physical/Chemical Data on Fluorochemical Products Related to Environmental Fate

The reliability code which follows the test value in parentheses is interpreted as follows:
(1A) Study used published test guidelines or well-documented procedures. Where applicable, concentrations were measured. All quality control data were acceptable.
(1B) Results were obtained by mathmatical estimation.
(2) Study meets all the criteria for quality testing, but has one or more deficiencies.
A. Concentrations NOT measured--parameter determined via indirect measurement.
B. Analytical methodology questionable.
(3) Study does **not** meet criteria for quality testing due to
A. Demonstrated weaknesses in experimental procedures.
B. Insufficient methodology description.
C. Unacceptable quality control.
(4) Study data are available only as summaries. Original reports unavailable.

N/D= not determined

Product Principle FC	Solubility in water mg/L	octanol/water partition coefficient	log n-octanol/water partition coefficient	soil adsorption coefficient (K)	organic carbon adsorption coefficient (K_{oc})	Vapor pressure
PFOS K^+ salt	1080 (2A)	10 (2B)	1 (2B)	0.99 (2B)	66 (2B)	N/D
N-MeFOSE alcohol	0.82 (2B)	56,800 (2B)	ND	77 (2B)	3,500 (2B)	N/D
N-EtFOSE alcohol	0.05 (2B)	6,600,000 (4)	3.60 (1B)	330 (2B)	17,800 (2B)	1.22 mmHg (1B) 0.5Pa@20°C (1A)
N-EtFOSEA	0.89 (2B)	N/D	>6 (2B)	N/D	N/D	6.0×10^{-3} Pa (1A)
POSF	1 est (2A)	N/D	N/D	N/D	N/D	1.6 torr@20°C (4)
N-EtFOSA	N/D	N/D	N/D	N/D	N/D	0.16 Pa@20°C (1A)

Historically, formulated products containing other components and residuals rather than pure perfluorochemicals were used to collect physical/chemical data. While most of the products above consist largely of one active fluorochemical component, the values obtained for the product are not likely those for the purified fluorochemical alone.

Computer models used in conjunction with empirical sampling can be used to predict environmental fate and transport of these substances. Existing models can require the following physical/chemical data for operation: molecular weight, boiling/melting point, pK_a, octanol/water partition coefficient, vapor pressure, solubility, Henry's law constant, density, evaporation rate, heat of vaporization, bioconcentration factor, and degradation mechanisms in air and water (hydrolysis, photolysis, and biodegradation). Precise values for the parent fluorochemical compound, its intermediates, and the end degradation product(s) are essential for comprehensive predictions about environmental fate and transport.

3M is developing the missing physical/chemical data on individual fluorochemicals with the assistance of several consultants. While laboratory studies are underway on physical/chemical properties of PFOS, EtFOSE alcohol and MeFOSE alcohol, models are being developed to estimate the physical/chemical properties of other sulfonated perfluorochemicals. The data are being determined using the Guidelines for the Testing of Chemicals developed by the Organization for Economic Co-operation and Development (OECD) for physical/chemical testing (3) where available. Where possible, melting points, boiling points, vapor pressures, dissociation constants, water solubility, n-octanol/water partition coefficients, air/water partition coefficients, and soil adsorption/desorption will be determined. This information is needed for both environmental fate models and manufacturing emission models. Current modeling efforts are hampered by lack of data on physical/chemical properties.

Data are being collected according to Good Laboratory Practice (GLP) standards. The air/water partition test is non-standard. This test protocol was developed jointly by 3M and an outside expert. Results will be reviewed by several technical experts, both within the 3M Environmental Laboratory, and outside the company.

3M is generating the information on soil sorption/desorption characteristics as non-GLP, screening studies. These data will aid in the evaluation of the transport process and partitioning. For example, will a fluorochemical be retained by the soil matrix or remain in the water phase? The bioconcentration potential of PFOS and EtFOSE alcohol will be examined through empirical testing that determines the extent of the uptake of these chemicals by fish. Work on degradation including hydrolysis, photodegradation and biodegradation is described in another section. (See Environmental Transformation/Degradation.)

The physical/chemical testing is proceeding in order of PFOS, EtFOSE alcohol, and MeFOSE alcohol. The results to date are reported in Table 4. The inability to determine an octanol/water partition coefficient makes it difficult to do predictive modeling.

Table 4. New Physical/Chemical Testing Results on PFOS, potassium salt

Parameter	Results
Solubility: pure water	570 mg/L
Solubility: fresh water	370 mg/L*
Solubility: unfiltered sea water	4-5 mg/L* estimated
Solubility: filtered sea water	25 mg/L*
Vapor Pressure	3.31×10^{-4} Pa @20°C
Melting Point	$\geq$ 400°C
Boiling Point	not calculable
Octanol/Water Partition (K_{ow})	not calculable; three phases
Air/Water Partition Coefficient	0($<2 \times 10^{-6}$)

*Data developed in support of other studies; not developed using GLP standards.

The methods used in these current physical/chemical tests will provide values reported in consistent formats that are internationally familiar and accepted. This standardization will aid in the review and comparison of data on individual fluorochemicals and in model operation and prediction. The data will contribute to analytical method development and overall improvements in sample handling, shipping and storage as well as manufacturing.

5.0 Analytical Test Methods for Fluorochemicals

Procedures for detecting and identifying fluorochemicals in the environment require a very high level of technical expertise. Most general analytical methods do not provide enough sensitivity or selectivity. The complex mixture of possible components in a product, the multiple matrices in which they could reside (e.g. the atmosphere, soils, surface water, groundwater, wastewater, different animal tissues, different animal species, plant species, foods, etc.), and trace level detection require selective extraction and diverse analytical techniques.

Each fluorochemical requires a unique analytical methodology. Separate methods may be needed for every matrix. Validation of each method is time intensive. Often, standards are not available. Reliable quantitative methods for extraction, separation and identification have been developed only within the last few years. Prior to that, relatively insensitive and non-specific analytical methods, such as "total organic fluoride (TOF)," were used.

The analytical technology used in extraction, separation, identification and quantitation includes combinations of:

- High Performance Liquid Chromatography (HPLC);
- High Pressure Solvent Extraction (HPSE);
- ElectroSpray Tandem Mass Spectroscopy (ESMSMS);
- Gas Chromatography (GC) with a Flame Ionization Detector (FID),
  a Mass Spectrometer (MS),
  a Photo Ionization Detector (PID), or
  an Electron Capture Detector (ECD)
- HPLC-Quadrapole- Time Of Flight-mass spectrometer (QTOF)

For example, analysis of PFOS extracted from tissues requires ESMSMS analysis. This technique focuses quantitation on three secondary ions of one primary ion at a specific HPLC retention time.

To provide positive identification of target analytes in complicated matrices, the 3M Environmental Laboratory uses a quadrapole time-of-flight mass spectrometer. The instrument provides high mass accuracy (to 0.0005 amu) and so is useful in identifying

fluorochemical metabolites and intermediates for which standards are not available. Compound identification is based on reasonable HPLC retention time as compared to standard compounds of similar structure, reasonable interpretation of fragment ions associated with the primary ion, interpretation of the accurate mass spectrum, and agreement between the experimental and theoretical molecular weight (+ 0.0005 amu).

The addition of new technology has permitted 3M analysts to increase the numbers of sulfonated perfluorochemicals that can be identified, expand the matrices in which they can be detected, and lower the levels at which they are detected. The technology has expanded the volumes of analyses that can be done. Nonetheless, capacity limits require analyses to be prioritized. When samples cannot be analyzed soon after collection, care is taken to store the samples appropriately for the matrix and the analytical method, both to prevent sample deterioration and contamination.

3M now has in place several methods for analysis of sulfonated perfluorochemicals in several matrices. The methods produce data of varying quality. They may be used in combination to produce test data. The method performance can be categorized as follows:

1. Quantitative methods that have been validated by studies conducted according to Good Laboratory Practices (GLP). These exist for analyses of samples of blood, liver, and several animal tissues of certain species, drinking water, and certain types of food.

2. Quantitative methods that typically are based on methodologies that have undergone significant analytical characterization during development. These methods are validated by extensive quality control testing, but validation studies may not have been conducted according to GLP requirements. These exist for wastewater, sludge, and air, for example.

3. Semi-quantitative methods that typically are based on the quantitative methods but for which validation studies are lacking or quality assurance cannot be demonstrated because, for example, standards are unobtainable or sample matrix is extremely limited.

4. Screening methods that typically are under development or a result of exploratory studies. These methods yield only qualitative data, i.e. they reliably detect the presence or absence of an analyte.

Method development is continuing, not only at the 3M Environmental Laboratory but also at independent laboratories in consultation with 3M Environmental Laboratory scientists. For some matrices, the detection limits sought are at lower levels. Method validation of low level analyses may be confirmed at a university or other contract laboratories, as appropriate. When samples are sent to consulting laboratories, 3M supplies the methodology or shares expertise to develop the method. Quality assurance is

required, along with method validation and oversight at levels comparable to those used in the 3M Environmental Laboratory.

Continual improvements are sought in analytical methods as the ability to detect trace quantities is essential for a number of reasons such as: screening laboratory supplies and environments prior to initiating toxicity testing, for detecting environmental exposure, for determining sources of perfluorochemicals, and for understanding perfluorochemical metabolism kinetics.

6.0 Sources of Fluorochemicals

A few fluorochemicals occur naturally in the biosphere, produced by biological and geochemical processes. Several green plants produce monofluoroacetic acid (CH_2FCOOH). Some fungi produce monofluorinated organics. All fluorochemicals produced biologically contain only one fluorine atom. Volcanoes and other geological processes produce tetrafluoroethylene, sulfur hexafluoride, perfluoromethane and some chlorofluorocarbons in small quantities.

Most fluorochemicals in the environment are present as a result of human manufacture and use. Releases of fluorochemicals into the environment can occur at each stage of the fluorochemical product's life cycle. They can be released when the fluorochemical is synthesized, continue during incorporation of the fluorochemical into a product, during the distribution of the product to users, during the use of the product by consumers, and during disposal practices at all of these stages.

3M is using a two step approach to estimate environmental releases of fluorochemicals. The initial efforts have focused on determining waste generated; the second step will focus on determining releases. This two step approach is necessary since not all waste produced will result in a release to the environment. Much of the waste that is generated is destroyed through treatment or otherwise actively managed to prevent release into the environment. Efforts are also being made to further tighten such controls.

3M has estimated waste generation from each of the following life cycle stages: the manufacturing processes, the supply and distribution chains, customer uses and product/waste disposal.

For ease in comparing waste stream data, wastes are described in term of "PFOS equivalents." PFOS equivalents are the weight of $C_8F_{17}SO_2$ present in a sulfonated perfluorochemical product. It is the mass of PFOS molecules that would be formed in the breakdown of the product. The assumptions of complete breakdown to PFOS of each sulfonated perfluorochemical product, in the year in which the product was sold, are unlikely "worst-case" assumptions. Various degradation testing finds a broad range of

product degradation rates. Some polymeric products appear to be quite stable in the environment, with long half-lives; other polymers hydrolyze quickly.

6.1 Manufacturing Waste Streams

The assessment of the release of sulfonated perfluorochemicals into the environment begins with manufacturing waste generation. Some waste streams, such as wastewater discharge or disposal of off-spec products, can be anticipated and controls provided. Other waste can be generated during any of the steps required to produce the fluorochemicals and manufacture the product.

The greatest production of the parent fluorochemical product, POSF, occurs at the Decatur, Alabama plant. Here POSF is created in electrochemical cells and undergoes numerous steps to convert it into final products. Salts of PFOS are also manufactured at the facility. Because of its production volume, the Decatur facility has been the focus of manufacturing waste stream studies. Understanding waste generation and how wastes are managed and disposed of provides a better understanding of potential releases into the environment. That understanding will help to identify opportunities for reductions in such releases.

The manufacturing process for sulfonated perfluorochemicals is complicated. There are more than 600 intermediate manufacturing steps associated with the production of POSF and POSF-based products. This translates into hundreds of process steps that require venting or that generate wastewater or solid waste. Although the manufacturing process attempts to capture, reuse, and recycle most fluorochemicals as desired product material, until recently, the unique chemistries created in each step of the process could not be analyzed precisely to confirm composition and to quantify amounts. The manufacturing process is dynamic, with rapidly changing matrices and many process steps. Ongoing process optimization activities continuously change the waste stream profile.

Progress has been made in analytical techniques. In 1997, analytical laboratory techniques and methods could quantitatively identify the presence of only one fluorochemical analyte in a wastewater matrix. In 1999, improved analytical techniques and methods were developed for additional fluorochemical analytes in a wastewater matrix.

Advanced field monitoring technology has been developed based on Fourier Transform Infrared spectroscopy (FTIR). This field tool has been used to detect where emissions to air are occurring during the manufacturing process and to evaluate whether a process change or a control technology can decrease the release.

As better analytical techniques become available, efforts are being made to:

- characterize the major manufacturing processes generating fluorochemical waste streams;
- evaluate the effectiveness of fluorochemical removal technologies; and
- provide better estimates of the amounts and kinds of fluorochemicals released to the environment from manufacturing processes and from waste treatment and disposal.

Information currently available on waste streams generated during manufacturing processes at Decatur is derived from engineering calculations, air emissions modeling, and limited testing. An overall site materials balance was developed in the mid- 1990's using the amount of POSF-based solids initially created in the electrochemical cell and the amount of POSF contained in final products sold. The difference was an estimate of *total* waste streams generated during processing. The emission factors derived from this balance are used to calculate waste streams from production throughput. They are the basis for the estimates in Table 5. These estimates derived from the material balance are not precise, as this methodology can produce only rough approximations.

The estimates in Table 5 reflect the most current information available and combine data derived from several sources: information from the mid-90s site balance, wastewater testing, waste disposal records, process models and supplemental information from 1997, 1998 and 1999. Several changes in waste disposal and processing have been implemented since the mid-1990s in order to reduce potential releases to the environment. Wastewater sludges that were once land applied on site are now sent to a municipal landfill for disposal. Off-spec materials that were discharged to wastewater are now shipped off-site to be incinerated.

Table 5 helps to demonstrate the vast difference between volumes of wastes generated and volumes of releases to the environment, since the vast majority of wastes sent to incineration are destroyed in the incineration process and most material sent off-site to landfills will be effectively managed to prevent release to the environment.

Table 5. Estimated 1998 Wastes Generated (in PFOS Equivalents) at the Decatur Manufacturing Plant

Waste Type	Estimated PFOS Equivalents, lbs
Air Emissions	19,000
Wastes sent off-site to Incineration	657,000
Wastes sent off-site to Landfills	380,000
Discharge to River after Wastewater Treatment	10,000
Total Wastes	1,066,000

Note: The 10,000 lbs/yr of PFOS equivalents in the discharge to the river are estimated releases to the environment after wastewater treatment, not the lbs/yr generated prior to treatment.

More explanation of the estimates and efforts currently underway in air, wastewater and waste management follows.

6.11 Waste Stream Characterization

Updating material balances for the manufacturing process is an ongoing effort. Today process engineers use a model of process steps to calculate air emissions. New information is being compiled to aid with model operation and waste calculations. The effort to determine physical/chemical properties for sulfonated perfluorochemicals will improve model inputs and waste stream calculations. Analytical technology is improving understanding of process chemistry

Data from the process engineers' available material balances in the plant's reporting system have been used to supplement the earlier site balance in estimating air emissions. Initial reports from this system indicate that most site waste and air emissions result from fewer than 10 key steps in the early stages of POSF production. Process experts are examining these steps for ways to reduce or eliminate the impurities and wastes generated in the steps.

In 1999, the Decatur plant installed a discotherm unit which heats the process materials, vaporizing and capturing the fluorochemicals. It will significantly reduce the organofluorides in the wastewater. This technology will operate to reduce emissions and waste at the source. It will make it easier to segregate waste streams and recycle fluorochemical wastes back into the process.

6.12 Air

3M engineers have reviewed specific process steps to determine what air emissions testing is feasible and appropriate. Testing of complex batch-processing systems is difficult due to quickly changing process conditions, venting pressures, and difficulty in isolating processes; however, characterization testing may be possible. The technical feasibility of performing this testing for two major processes is now under evaluation. Any emissions testing will require modifications to process vents and mitigation of potential safety hazards. About 80 separate venting points are associated with the equipment used to make sulfonated perfluorochemicals.

6.13 Wastewater

Analytical methods have been developed during the past year to better characterize the wastewater discharge from the site. The first testing of wastewater before and after treatment for specific fluorochemicals occurred at Decatur early in 1998. The testing was

limited and reflected operating conditions for a relatively short period of time (24 hour composite samples of influent and effluent for one week.) Some of the compounds that were identified in the wastewater were: a diester of EtFOSE alcohol, EtFOSE alcohol, MeFOSE alcohol, PFOS, FOSA, PFOSAA, PFOA and PFHS.

In 1998 an interim carbon adsorption treatment system was installed as part of wastewater treatment. Data for the effluent estimate in Table 5 reflects this change. This treatment system treats the largest single source of fluorochemical-containing wastewater in order to remove PFOS and other sulfonated perfluorochemicals from the wastewater. Comparison of the results from sampling done in February 1998 with sampling done in the end of 1998 indicates the quantity of PFOS discharged to the Tennessee River declined by about half. In addition to the carbon adsorption system, in-process operational changes were made in off-spec product discharge procedures that also contributed to the reduction in PFOS content of the discharge to the river.

The carbon system has been incorporated as a permanent upgrade of the wastewater treatment system. Monitoring indicates that with proper operation, carbon adsorption removes better than 99% of PFOS. Removal efficiency of other sulfonated perfluorochemicals varies, but the treatment appears to provide a high degree of removal for most. A number of wastewater streams currently going to sewers are in the process of being diverted to thermal treatment facilities for disposal. This will result in a reduction in the values listed in Table 5. 3M has conducted an extensive review of state-of-the-art technology for wastewater treatment. Various upgrades are currently being evaluated. The long term goal of wastewater treatment at the plant is to utilize source control and end-of-pipe treatment to remove nearly all sulfonated perfluorochemicals from wastewater prior to discharge to the river.

6.14 Solid Waste

An effort to identify all waste streams and their disposal methods is underway. Existing waste tracking is done on a site basis, so it is difficult to distinguish the particular streams with POSF chemistry. The mid-1990s emission estimates did not distinguish final disposal of the material lost from production, so site records were used in combination with the existing emission estimates to create the current picture of potential releases resulting from disposal.

A review of plant records for 1998 has been completed to determine primary waste disposal locations for the site. According to Decatur plant records, 63% of the fluorochemical containing wastes are sent to incinerators, 33% of the wastes are disposed in hazardous waste landfills and 4% in non-hazardous waste landfills.

6.2 Supply Chain Waste Streams

Using sales data, 3M identified key products that contain a majority of the fluorochemical solids used in products. These products represent 89% of PFOS-equivalents sold by 3M in 1997 in the United States. Most commonly, these products were sold to commercial users who applied them or incorporated them into their products.

Using the information developed from sales, 3M estimated customer and end user waste streams (Table 6). These estimates are imprecise and based on several assumptions, but provide qualitative information. Using the chemical formula for PFOS, the fluorochemical solids were converted to "PFOS equivalents" for ease in estimating and comparing total losses of sulfonated perfluorochemicals and in comparing losses. The assumptions of complete breakdown to PFOS of each sulfonated perfluorochemical product, in the year in which the product was sold, are unlikely "worst case" assumptions. Product waste stream estimates are based on conservative, worst case assumptions about the generation of waste streams at supply chain facilities. These are often based on operator experience or engineering estimates rather than laboratory tests and can result in wide ranges in waste stream calculations. In estimating wastes, these data do not include loss of product residuals in the waste streams because information on the properties of residuals and processes at supply chain facilities and end user locations is inadequate to estimate this loss.

Initial estimates associate waste streams generated from uses and disposal of the products by customers of each business unit. These estimates are helping to focus efforts in improving customer stewardship practices and 3M product reengineering. As is evident, most of the waste generated is in the form of solid waste.

Table 6. Customer and End User Waste Stream Estimates, PFOS equivalents, lbs in 1997

Waste Stream	Supply Chain	Use	Disposal
Air	2,600	3,300	0
Wastewater	112,000	181,000	0
Solid Waste	59,000	377,000	1,262,000

6.3 Releases from Waste Treatment and Disposal Methods

3M and its consultant are gathering information on treatment and waste handling at several landfills and wastewater treatment plants which receive wastes containing sulfonated perfluorochemicals from the supply chain facilities and 3M manufacturing

facilities. Information is also being compiled on some of the largest wastewater treatment facilities and landfills in the United States in order to estimate the potential perfluorochemical releases to the environment from municipal disposal facilities not associated with the supply chain or manufacturing.

Incineration is a favored disposal method because of its high rates of destruction of sulfonated compounds. 3M and its consultant are further evaluating the effectiveness of incineration for this purpose. The basic bond breaking chemistry of thermal destruction of POSF-based fluorochemicals, the destruction efficiencies of various technologies/situations such as municipal incinerators, and the products that could result from incomplete combustion are elements of the study. The study involves a review of 3M and external literature to compile information on the formation and properties of thermal transformation products of sulfonated perfluorochemicals.

Modeling will be used to determine to the extent practical, the releases to the environment from the amount of material sent to incineration, wastewater treatment plants, and landfills.

The goals of the life cycle release studies are:

- to identify important fluorochemicals based on volume of release, mode of release and chemistry;
- to provide values for use in modeling the distribution of fluorochemicals in the environment;
- to determine sampling sites and substantiate sampling results;
- to predict which fluorochemical releases may result in exposure to humans and the environment; and
- to identify fluorochemicals that require further study as to their transport, fate and exposure potential.

7.0 Environmental Transport and Distribution

The transport and fate of chemicals in the environment depends on many factors but principally on the interaction between environmental conditions (e.g. water, temperature, sunlight), and chemical properties (e.g. partitioning and reactivity). In the environmental area, eleven important fate and transport mechanisms for sulfonated perfluorochemicals have been identified for further study. These are:

1. Partitioning between air and product, i.e. volatilization from product to air;
2. Indoor air deposition;
3. Accumulation on airborne particulates;
4. Fate and transport to the stratosphere;

5. Accumulation at the surface water microlayer;
6. Degradation (includes hydrolysis, photolysis and biodegradation);
7. Dissociation in water;
8. Uptake in plants;
9. Uptake in fish;
10. Uptake in birds;
11. Efficiency of wastewater treatment systems.

All of these fate and transport mechanisms have been linked to models. Modeling uses mathematical equations to simulate and predict real events and processes. Many types of models will be considered for use in this effort to evaluate sulfonated perfluorochemicals. Simple models of ecosystems, indoor air, and treatment systems (wastewater, landfills) are being used to screen for possible fate mechanisms, possible exposures, and possible sample detection limits. For example, one preliminary screening model suggests that top trophic level species such as fish eating birds and sea mammals should be examined. This finding was incorporated into the design of the biosphere sampling plan.

Chemicals differ greatly in their behavior. The major differences in behavior of organic chemicals in the environment are due to physical-chemical properties. Although laboratory studies are underway on physical/chemical properties of PFOS, EtFOSE alcohol and MeFOSE alcohol, models are being developed to estimate the physical/chemical properties of other sulfonated perfluorochemicals. This will reduce the time and testing required to gather these data for use in environmental fate models.

Fugacity is a concept that is used to describe the tendency of a compound to migrate in and between one environmental medium and another. Different media include air, water, soil, sediment, and biota, all of which together compose a dynamic, interactive system--an *eco*system. Predictions about movement of a chemical must incorporate both its physical/chemical properties and the environment the chemical is in. For example, a low vapor pressure does not mean a chemical is not present in air. It may evaporate appreciably from water despite a low vapor pressure if it has low solubility in water. By entering the physical-chemical property data on a chemical into a fugacity model of a generic or specific environment, it is possible to estimate general features of a chemical's likely behavior and fate. The output of these calculations can be presented numerically and pictorially. (6)

Fugacity models will be used to predict fate and transport of sulfonated perfluorochemicals. Existing fugacity models typically are based on experience with chlorinated organics. An internationally recognized modeling expert is developing/ adapting models to consider the unique properties of fluorochemicals. The goal of this modeling effort is to have a multimedia model or models to predict the fate of sulfonated perfluorochemical products and associated byproducts in a variety of ecosystems.

8.0 Environmental Sampling for Fluorochemicals

8.1 Environmental Levels

8.11 Historical Data

In the late 1970s, 3M conducted a very limited number of studies to assess the distribution of fluorochemical constituents in the environment. Several freshwater fish species were tested for a number of fluorochemical compounds. In reviewing the data obtained from these studies in context of the current knowledge of the behavior of these materials, 3M has concluded that these historical data are highly questionable and may be misleading. Therefore, they are not included in this paper. The sections following present more reliable data and information collected using validated sampling and analytical methodologies.

8.12 Recent Analyses of Wild Birds and Fish

In analysis in 1999 of the plasma of ten fish eating birds, albatross nestlings at Midway Island in the Pacific Ocean and eagle nestlings in Minnesota and Michigan, PFOS was detected in each of the samples from eagles. The samples were collected in 1989, 92, and 93 by Dr. John Giesy of Michigan State University as part of other surveys. Three of the albatross adults showed no detectable levels of PFOS (< 1 ppb detection level). Detectable, but not quantifiable levels of PFOS were found in the remaining albatross samples, both collected from birds less than a year old. All albatross samples were collected in 1992-93. See Table 7. These data are semi-quantitative, screening quality. As only a small amount (< 1mL) of plasma was available to conduct the analyses, no matrix spikes were possible to estimate the method's recovery efficiency, but the methods used have been characterized in other, similar matrices.

After the initial screening results on wild bird plasma, the plasma from a second set of wild birds was examined for the presence of PFOS. (See Table 7.) The source of the plasma was three sea eagles collected from the Baltic Sea and seven bald eagles collected from North America. The samples were collected in 1992-93 and again by Dr. John Giesy. PFOS was detected in all of the eagle plasma screened. These data are semi-quantitative, screening quality. Two matrix spikes (250 ppb) prepared from eagle plasma were extracted and analyzed. Both showed >80% recovery.

Table 7. Levels of PFOS in the Plasma of Wild Birds

Species	Collection Date	Location	Age, Gender	PFOS, ppb
Bald Eagle	5 Jun 93	Lower Penn, MI	163 days, F	30
Bald Eagle	3 Jun 93	Lower Penn,MI	228, F	34
Bald Eagle	1989	Upper Penn, MI	unknown	77
Bald Eagle	1989	Upper Penn, MI	unknown	31
Bald Eagle	17 Jun 92	Voyageurs,MN	82 days, M	34
Albatross	13 Dec 92	Midway atoll	6 years	BLD
Albatross	18 May 93	Midway atoll	0	BLQ
Albatross	13 Dec 92	Midway atoll	8 years	BLD
Albatross	13 Dec 92	Midway atoll	15 years	BLD
Albatross	18 May 93	Midway atoll	0	BLQ
Sea Eagle	28 May 93	Baltic, Sweden	nestling	125
Sea Eagle	27 May 93	Baltic, Sweden	nestling	93
Sea Eagle	23 May 93	Baltic, Sweden	nestling	215
Bald Eagle	26 Jun 92	North America	nestling	165
Bald Eagle	28 Jun 93	North America	nestling	198
Bald Eagle	23 Jun 92	L. Superior ONT	nestling, F	494
Bald Eagle	5 Jun 92	North America	Adult, F	1047
Bald Eagle	26 Jun 92	Devil's Is., WI	nestling, F	226
Bald Eagle	22 Jun 92	Mud Creek,OH	nestling,	371
Bald Eagle	8 Jun 92	Carroll Twp, OH	nestling	374

BLQ= Below Limit of Quantitation (10 ppb)
BLD= Below Limit of Detection (approximately 1 ppb)

Following the bird plasma studies, sixty liver samples collected by the U.S. Fish & Wildlife Service from various species of birds were analyzed. The dead birds were collected at a variety of sites across the United States. They were not part of a controlled research study, but were selected for their location and diet. All but sandhill cranes are fish eating species. The sandhill cranes are an insect eating species. The purpose of the analyses was to determine if the presence of PFOS could be detected in these sample matrices. 3M believes that these sets of data are insufficient to draw conclusions with any statistical merit. The PFOS data in Table 8 are semi-quantitative, screening quality, with a margin of error estimated at $\pm$ 30%. The limit of quantitation for PFOS is 6 ppb.

Table 8. Analysis of Wild Bird Livers.

BLQ= Below limit of quantitation (6 ppb)

Sample No.	Species	Location	PFOS ppb
1	Sandhill Crane	Kearney, NE	41
2	Sandhill Crane	Kearney, NE	BLQ
3	Sandhill Crane	Kearney, NE	BLQ
4	Sandhill Crane	Kearney, NE	BLQ
5	Sandhill Crane	Kearney, NE	BLQ
6	Sandhill Crane	Chochise Co., AZ	BLQ
7	Sandhill Crane	Chochise Co., AZ	BLQ
8	Sandhill Crane	Chochise Co., AZ	BLQ
9	Sandhill Crane	Chochise Co., AZ	BLQ
10	Sandhill Crane	Chochise Co., AZ	BLQ
11	White Pelican	Calipatria, CA	35
12	White Pelican	Calipatria, CA	1293
13	White Pelican	Calipatria, CA	29
14	White Pelican	Calipatria, CA	15
15	White Pelican	Calipatria, CA	153
16	Brandt's Cormorant	San Diego, CA	53
17	Brandt's Cormorant	San Diego, CA	46
18	Brandt's Cormorant	San Diego, CA	46
19	Brandt's Cormorant	San Diego, CA	80
20	Brandt's Cormorant	San Diego, CA	2055
21	Dbl. Crested Cormorant	St. Martinville, LA	59
22	Dbl. Crested Cormorant	St. Martinville, LA	145
23	Dbl. Crested Cormorant	St. Martinville, LA	333
24	Dbl. Crested Cormorant	St. Martinville, LA	76
25	Dbl. Crested Cormorant	St. Martinville, LA	170
26	Brown Pelican	Miami, FL	106
27	Brown Pelican	Miami, FL	134
28	Brown Pelican	Miami, FL	125
29	Brown Pelican	Miami, FL	159
30	Brown Pelican	Miami, FL	48
31	Sandhill Crane	Valenica Co., NM	BLQ
32	Sandhill Crane	Valenica Co., NM	BLQ
33	Sandhill Crane	Socorro Co., NM	BLQ
34	Sandhill Crane	Socorro Co., NM	BLQ
35	Sandhill Crane	Valenica Co., NM	BLQ
36	Dbl. Crested Cormorant	Naples, FL	212
37	Dbl. Crested Cormorant	Naples, FL	10
38	Dbl. Crested Cormorant	Naples, FL	52
39	Dbl. Crested Cormorant	Naples, FL	100
40	Dbl. Crested Cormorant	Naples, FL	152
41	Brown Pelican	Calipatria, CA	16
42	Brown Pelican	Calipatria, CA	36
43	Brown Pelican	Calipatria, CA	BLQ
44	Brown Pelican	Calipatria, CA	6
45	Brown Pelican	Calipatria, CA	32

Sample No.	Species	Location	PFOS ppb
46	Great Blue Heron	St. Martinville, LA	188
47	Great Blue Heron	St. Martinville, LA	59
48	Great Blue Heron	St. Martinville, LA	1061
49	Great Blue Heron	St. Martinville, LA	261
50	Great Blue Heron	St. Martinville, LA	173
51	White Pelican	Fallon, NV	141
52	White Pelican	Fallon, NV	362
53	White Pelican	Fallon, NV	927
54	White Pelican	Fallon, NV	133
55	White Pelican	Fallon, NV	291
56	Brown Pelican	Ft. Lauderdale, FL	194
57	Brown Pelican	Ft. Lauderdale, FL	75
58	Brown Pelican	Ft. Lauderdale, FL	71
59	Brown Pelican	Ft. Lauderdale, FL	31
60	Brown Pelican	Ft. Lauderdale, FL	91

In addition to wild birds, some fish from the wild were tested for the presence of PFOS. The fish were collected in 1997-98 from sites in Michigan as part of surveys conducted by Dr. John Giesy. They were stored frozen and analyzed in 1999. Six species were tested. Low levels of PFOS were detected in four of the twelve samples. Since no sample matrices were available for matrix spike studies, these data are of screening quality only. No clear meaning can be drawn from the data. They are being used to develop sampling programs. Table 9 reports the findings.

Table 9. PFOS Screening in Fish.

BLD= Below Limit of Detection (approximately 7ppb)
BLQ=Below Limit of Quantitation (approximately 70 ppb)

Sample No.	Species	Location	Test Matrix	Test Result
1	Carp	Pine River, MI	whole body	BLD
2	Lake Trout	Siskiwit Lake, Isle Royale, MI	whole body	BLD
3	Lake Trout	Siskiwit Lake, Isle Royale, MI	whole body	BLD
4	Lake Trout	Pine River, MI	whole body	BLQ
5	Lake Trout	Lake Superior	whole body	BLD
6	Walleye	Detroit River, MI	whole body	BLD
7	Ciscowet	Lake Superior, Marquette, MI	muscle	BLD
8	Brown Trout	Detroit River, MI	muscle	BLD
9	Brown Trout	Rouge River, MI	liver	BLQ
10	Channel Catfish	Lake St. Claire, MI	muscle	BLD
11	Channel Catfish	Lake St. Claire, MI	egg	BLQ
12	Channel Catfish	Lake St. Claire, MI	egg	BLQ

8.13 Testing of Fishmeal Used in Rat Studies

While performing human health toxicity studies (see Perfluorooctane Sulfonate: Current Summary of Human Sera, Health and Toxicology Data, January 1999), 3M found "endogenous" levels of PFOS in some of the naive rats used in the studies. The levels found in the rat livers ranged from 29 ppb to 300 ppb. Livers of rats from one supplier showed no PFOS above the detection limit of 15 ppb. Further investigation revealed fishmeal to be an ingredient in the rat chow fed to the rats in which PFOS was detected. Fishmeal was not a dietary component of the rats that had no detectable levels of PFOS. 3M developed a complex analytical method to analyze fishmeal samples collected from different fish stock. At a detection limit of 2 ppm, PFOS was detected in three samples of fishmeal and not detected in three samples. At this time, these data are not conclusive.

8.14 Plant Site Analyses

In March of 1998, 3M conducted screening level sampling for PFOS around the Decatur plant. The outfall of the Decatur wastewater treatment plant is at a bay near the mouth of Baker's Creek. Baker's Creek flows into the Tennessee River, a large river that supports barge traffic. About 25 miles downstream is Wheeler Dam. The samples tested were of water surface film, subsurface water and sediment. A goal of the sampling was to experiment with sampling techniques and analytical methods. Therefore, the analytical data are of screening quality only. Data on PFOS from the sampling are in Table 10.

Table 10. Sampling Near the Decatur Wastewater Discharge

Sample Locations:
UP1 & UP2: Tennessee River, upstream of discharge
BC1: Baker's Creek below outfall
Q1 & Q2: Baker's Creek, downstream of discharge, in quiet waters near Tennessee River
WD1 & WD2: Tennessee River below Wheeler Dam

	UP1	UP2	BC1	Q1	Q2	WD1	WD2
Sub-surface water, in ppm							
PFOS	<.010	<.010	0.44	0.025	0.012	<.010	<.010
PFOS homologues	<.010	<.010	0.10	<.010	<.010	<.010	<.010
Surface films, in ppm							
PFOS	N/C	N/C	1.60	1.00	0.28	N/C	N/C
PFOS homologues	N/C	N/C	0.02	<.010	<.010	N/C	N/C
Sediment, in ppm							
PFOS	0.177	<.050	31.1	N/C	N/C	<.050	<.050
PFOS homologues	<.050	<.050	<.050	N/C	N/C	<.050	<.050

N/C = not collected
Surface film samples were skimmed from the top of the water, at the air/water interface.
Sediment samples were collected from the river bed using an Ekman Dredge. Samples were taken at the water collection point or, if sediment was lacking there, as close as possible to it.

Based on this initial sampling, a more extensive sampling was conducted. Sampling locations extended from about 10 miles upstream of the facility to 25 miles below the facility. As a result of analytical techniques being developed to lower detection limits, analyses of these samples is pending.

8.15 Biosphere Sampling

3M is building on recent information with advances in technology to design a program that could detect traces of sulfonated perfluorochemicals across a range of species, environmental habitats and geographic locations, including soil, water and organisms. 3M's approach is to use existing, scientifically recognized, sampling and data collection programs in order to minimize the time needed to obtain information. The goal is to set some bounds on the geographic regions where sulfonated perfluorochemicals are currently found, identify areas that should receive more investigation, and eliminate some general environments from further sampling in the immediate future. Key ecosystems and species of concern surrounding manufacturing plants are being tested as well as ecosystems remote from manufacturing and use locations.

Where possible, synoptic samples of soil, sediment, air or water are also being taken, but the primary focus of initial studies is tissue samples from biological receptors, especially those in upper trophic levels. The information obtained in the initial studies will be used to determine appropriate studies for ascertaining critical pathways.

8.2 Human Exposure Levels

Studies to investigate human exposures take several approaches:

1. Environmental exposure of the general U.S. population will be assessed in phases through a "Multi-Cities Study." This involves field investigation of paired cities, one with significant manufacturing or commercial fluorochemical use, matched with a city without known significant use. The study will involve direct sampling for dietary and environmental presence.

2. Residential exposure will be assessed through a product's use and controlled measurements of the product's releases. This study will measure releases of fluorochemical residuals and total PFOS from carpets.

3. The migration of sulfonated perfluorochemicals used in food packaging to the food is being quantified for several foods.

8.21 Multi-cities Sampling

The multi-cities study pairs a city having significant manufacturing or commercial use of fluorochemical products based on customer sales with a city that does not. Initially six cities, (three pairs) are being examined. This may be expanded, depending on initial results. The multi-cities sampling will yield environmental distribution data as well as data on potential sources of human exposure. The cities were selected to represent urban locations with various levels of fluorochemical releases and various types of municipal water supplies. The samples to be obtained, where possible, are: urban air, surface water column and surface microlayer, sediment, river fish, drinking water intake, treated drinking water, tap water, the influent and effluent to publicly owned waste treatment works, sludge, and municipal landfill leachate. Additionally a "market basket" of several food products will be sampled. These include: beef, pork, chicken, hot dogs, catfish, eggs, milk, bread, green beans, apples from three grocery stores and, if possible, produce from local farmers' markets.

8.22 Carpet Use Studies

The carpet study will estimate any loss of fluorochemical from normal use of carpets. If a pilot study of carpets finds significant releases, then the study will assess human exposure that may occur via inhalation, dermal and ingestion routes.

8.23 Paper and Packaging Studies

Results of past studies on the migration of fluorochemicals from packaging into food have been submitted to the FDA, and FDA has cleared the use of paper and packaging protectors for food as indirect food additives. Current work focuses on the development of new methodologies to extract various fluorochemicals from paper and several foods, then perform quantitative, low level analyses (< 1 ppb).

8.24 Exposure Scenarios

These scenarios will be developed using data from release, fate and distribution studies. Their purpose is to prioritize exposure pathways for further study by developing quantitative estimates of specific exposures under known conditions in a specific location.

9.0 Environmental Transformation/Degradation of Fluorochemicals

There are many physical, chemical and biological mechanisms that operate in the environment to transform or degrade molecules. They include abiotic mechanisms, e.g. hydrolysis and photolysis, and biotic mechanisms, especially microbial metabolism. Because the carbon-fluorine bond is one of the strongest in nature, with high bond energies, its cleavage requires large amounts of energy. Most chemical and physical processes naturally occurring in the biosphere lack the required energy. In the laboratory, perfluoroalkyl chains are not degraded in the chemical oxygen demand (COD) test, nor in total organic carbon (TOC) analyzers that use very reactive chemical and ultraviolet degradation mechanisms. Combustion does destroy organic fluorochemicals and degradation is found in high temperature TOC analyzers.

In perfluorinated molecules, the fluorines surround the carbon chain completely, shielding the carbon-carbon bonds from attack. The fluorine atoms confer a "rigidity" to the conformation of the molecule. This rigidity could make it difficult for the molecule to join with enzymes, thereby blocking biological attack of the carbon-carbon bond. As a molecule becomes more fluorinated, carbon-carbon bonds, carbon-hydrogen and carbon-fluorine bonds all typically increase in strength.

Early work with perfluorochemical products using standardized screening tests for degradation found little susceptibility to degradation. (See Table 11.) Fluorochemicals lacking nonfluorinated organic portions produced essentially no biochemical oxygen demand (BOD). Those with ionically bonded organics showed BODs near those expected from their non-fluorinated portion alone. Fluorochemical surfactants with covalently bonded organic portions produced mixed results.

The early data on these degradability studies has been given a reliability code that follows the test results.

Table 11. Historical Results of Standard Degradation Tests on Fluorochemicals

Product Principle Fluorochemical	COD mg/Kg	BOD 5-day mg/Kg	BOD 10-day mg/Kg	BOD 20-day mg/Kg	BOD 28-day mg/Kg	Photo degra-dation	Other
POSF	500-720 (4)	nil (4)	nil (4)	nil (4)	N/D	N/D	N/D
N-MeFOSE alcohol	163,000 (1)	N/D	N/D	nil (1)	N/D	nil (1)	N/D
N-EtFOSE alcohol	260,000 (4)	nil (4)	N/D	N/D	N/D	nil (1)	O2 uptake= 3% of ThOD; No deg in 6 month shake flask studies or 7 day activated sludge studies (2B)
N-EtFOSA	1,800 (4)	nil (4)	nil (4)	nil (4)	N/D	N/D	N/D
N-EtFOSEA	240,000 (1)	12,000 (2A)	19,000 (2A)	23,000 (2A)	N/D	N/D	not readily (1) biodegradable
N-EtFOSEMA	80,000 (1)	800 (2A)	2,000 (2A)	11,000 (2A)	N/D	N/D	N/D
Perfluoro C10, sulfonic acid, NH4 salt	1,000,000 (2A)	67,000 (2A)	600,000 (2A)	720,000 (2A)	N/D	N/D	N/D
K salt of carboxylic acid analogue of N-EtFOSE alcohol	462,000 (1)	<39,800 (2A)	172,000 (2A)	179,000 (2A)	289,000 (2A)	N/D	N/D
PFOS Li^+ salt (4)	54,000	N/D	N/D	N/D	N/D	N/D	N/D
PFOS K salt	4,000 (4)	nil (4)	nil (4)	nil (4)	N/D	nil (4)	no degradation in Warburg 3 hr study or 2.5 month shake flask study (2B)
PFOS DEA salt	78,000 (2A)	44,000 (2A)		82,000 (2A)	N/D	N/D	N/D
N-EtFOSE alcohol $(C_2H_4O)_{14}H$ adduct	1,070,000 (1)	0 (4)	N/D	107,000 (4)	N/D	N/D	40% removal BiAS (3A)
PFOS NH_4 salt	412,000 (4)	232,500 (4)	267,500 (4)	292,500 (4)	N/D	N/D	N/D

COD means Chemical Oxygen Demand. It is a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant such as potassium dichromate.

BOD means Biochemical Oxygen Demand. It is the amount of oxygen consumed by microbial processes while breaking down a known amount of a test substance.

ThOD means Theoretical Oxygen Demand. It is the theoretical quantity of oxygen used when the test compound is fully mineralized. This value is calculated using the structure of the test chemical.

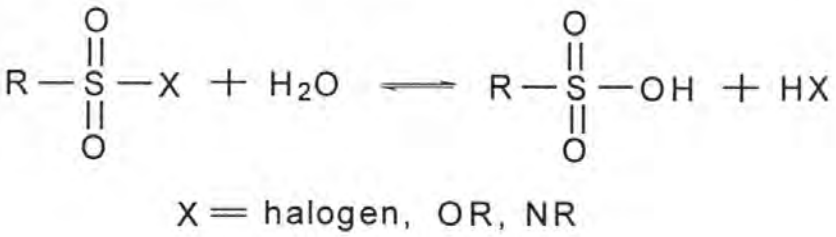
BiAS means Bismuth Active Substances. These are materials, such as water soluble polyethoxylates, that precipitate with barium tetraiodobismuthate.

N/D means Not Determined.

Code meanings are:
(1) Study used published test guidelines or well-documented procedures. Concentrations were measured, and all quality control data were acceptable.
(2) Study meets all the criteria for quality testing but has a deficiency
    A. Concentrations NOT measured.
    B. Analytical methodology questionable.
(3) Study does NOT meet criteria for quality testing; data have one or more flaws.
    A. Demonstrated weakness in experimental procedures.
    B. Insufficient description of method.
    C. Unacceptable performance of controls.
(4) Data are available only as summaries; original reports not found.

9.1 Hydrolysis Studies

Hydrolysis is a major mechanism contributing to abiotic degradation of organic molecules, although it rarely is responsible for complete degradation. The hydrolysis of sulfonated compounds is described below.



3M is evaluating the potential for hydrolysis of fluorochemicals using EPA guidance [Fate, Transport and Transformation Test Guidelines, *Hydrolysis as a Function of pH and Temperature*](2), and is conducting pH dependent studies of PFOS and MeFOSE alcohol, as well as on fluorochemical monomers, to estimate half-lives. Selected fluorochemical products are being subjected to a single temperature (50°C), variable pH screening process. For those that demonstrate hydrolysis or a deviation form first order kinetics, multiple pH, multiple temperature studies are planned. Hydrolysis test data are being reviewed by an outside expert.

9.2 Photolysis

Like hydrolysis, photodegradation is a major abiotic mechanism contributing to the transformation of organic molecules, but rarely responsible for complete degradation. Photodegradation occurs primarily in air, in shallow water, on soil and vegetative surfaces. It is likely an important factor in the fate of soluble and volatile compounds, less so for insoluble and sorbed compounds. Products and intermediates most susceptible to photodegradation are those most likely to be used outdoors in sunlight.

Initially, the degradation that might occur in products dissolved or suspended in water is under investigation. The first studies are using the PFOS precursors such as EtFOSE alcohol, MeFOSE alcohol, MeFOSA, EtFOSA, and FOSA. Later studies will use POSF-based polymers. If simple methods can be found, gas phase photolysis of volatile and semi-volatile fluorochemical degradation intermediates will be investigated.

The preliminary results suggest that PFOS is unchanged as a result of light exposure. However, EtFOSE alcohol, MeFOSE alcohol, EtFOSA and MeFOSA as well as a surfactant and foamer product all appeared to undergo photolysis to FOSA, PFOA, a hydride, and olefins. PFOS was not detected. One product, an aromatic perfluorooctane sulfonate, did photodegrade to form PFOS.

9.3 Atmospheric Studies

Although PFOS has a low volatility, several PFOS precursors are volatile. These include: EtFOSE alcohol, MeFOSE alcohol, MeFOSA, EtFOSA, and FOSA. When present as residuals in products, these precursors could evaporate into the atmosphere when the product is sprayed and then dried. Once in the atmosphere, the compounds can remain in the gas phase, condense on particulates present in the atmosphere and be carried or settle out with them, or be washed out with rain. The measured vapor pressure of Et-FOSE alcohol is sufficiently high that essentially all of it is likely to be in the gas phase and not condensed on particulate matter. Gas chromatic data suggest that other precursors are even more volatile. The low water solubility of these compounds makes it unlikely they washout from the atmosphere in rainwater.

Thus the rate of removal of these precursors from the atmosphere will likely depend on their photochemical reactivity, e.g. their reaction with hydroxyl ions in the atmosphere. How widely distributed they are locally, regionally or globally depends on the rate of photochemical transformation to more soluble or less volatile products.

3M is examining atmospheric lifetimes of these PFOS precursors. Initially EtFOSE alcohol and MeFOSE alcohol will be tested for reactivity with the OH radical in the gas phase. Modeling will determine their atmospheric lifetimes and analytical work will determine their gas-phase degradation products. Then those properties of the degradation

products that affect removal rates from the atmosphere, e.g. solubility and vapor pressure, will be determined. This information will be used to predict distribution of these compounds resulting from atmospheric mechanisms.

9.4 Biodegradation Studies

Biodegradation is essential to the functioning of living systems. Natural systems rely on living organisms, especially microbes, to break down complex organic molecules to simple inorganic molecules that can be recycled back into the ecosystem. Some microbial communities have demonstrated the ability to degrade some xenobiotic compounds. During biologically catalyzed degradation of these compounds, the degradation intermediates produced are frequently of a molecular structure that naturally occurs. Particularly important environments for biological breakdown are: sewage treatment systems, soils/sediments, estuaries and wetlands. Both aerobic and anaerobic organisms play important roles in degradation. 3M is studying biodegradation using several approaches.

9.41 Microbial Studies on Perfluorochemicals

Work at Michigan State University by Blake Key (6,7) under the direction of Dr. Craig Criddle used a laboratory isolate of a bacterium, a *Pseudomonas* species, to investigate the potential for biodegradation of fluorinated sulfonates. The researchers used model fluorinated sulfonate compounds: difluoromethane sulfonate (DFMS), trifluoromethane sulfonate (TFMS), 2,2,2-trifluoroethanesulfonate (TES), PFOS and H-PFOS (1H,1H,2H,2H-perfluorooctane sulfonate).

Criddle et al. demonstrated that the microorganism degraded those fluorochemical compounds containing hydrogen and used them as sulfur sources for growth under sulfur-limiting, aerobic conditions. They later found that such degradation occurred in soil even when sulfur was not limiting. The organism completely defluorinated DFMS. It used DFMS as the sole source of sulfur, but not as a source of carbon or energy. TES and H-PFOS were partially defluorinated. Six volatile products were detected for H-PFOS, all containing oxygen and fluorine but not sulfur. Where the carbons were fully fluorinated, i.e. TFMS and PFOS, no degradation was found. Criddle et al. concluded that the transformation of fluorinated sulfonates required the presence of hydrogen at the alpha carbon on the fluorinated alkyl chain. They theorized that when hydrogen is present at the alpha carbon, a site for attack is provided and the carbon-sulfur bond becomes more accessible. Perfluorinated compounds have a rigidity conferred by the fluorine substitution and no structures that are susceptible to electrophilic or nucleophilic attack.

9.42 Biological Transformation

When perfluorinated organic molecules do biodegrade, it is not the fluorinated portion that is affected. Enzymes attack at non-fluorinated side chains. Rather than complete degradation, i.e. degradation to inorganic compounds, another fluorinated molecule results from biodegradation processes. Existing studies of metabolism appear to indicate that for POSF-based compounds, the biological degradation halts when PFOS is formed.

$C_8F_{17}SO_2\text{-}F, + H_2O \longrightarrow C_8F_{17}SO_3^-$

POSF **PFOS**

$C_8F_{17}SO_2\text{-}R \longrightarrow \longrightarrow \longrightarrow C_8F_{17}SO_3^-$

POSF derivative **PFOS**

Once formed, PFOS has not been shown to degrade any further under any natural conditions except combustion. Because PFOS is resistant to physical, chemical and biological degradation, it persists in the environment, but the mechanism of accumulation is under study.

9.43 Optimizing Conditions for Biodegradation

Past studies on fluorochemicals with hydrocarbon portions have demonstrated resistance to biodegradation under standard test conditions, i.e. aerobic microbial degradation using a wastewater inoculum. These studies did not examine all combinations of conditions that could be optimized to favor the degradation of partially fluorinated chemicals.

3M is conducting new screening studies for biodegradation. These will determine if aerobic and/or anaerobic degradation of key fluorochemicals occurs using activated sludge, anaerobic sludge, aquatic sediments and soil. If degradation occurs, the studies will determine to what extent it occurs and the nature of degradation products. It will also provide information on the degree of fluorochemical sorption onto microbial sludges and toxicity to microbes. New studies are being designed to promote degradation. They will use enriched environments that support biodegradation, e.g. sewage, soil, sediments, and cultures of microbes selected for biodegradation capabilities.

10.0 Ecotoxicity Testing of Fluorochemicals

Ecotoxicology is the extension of toxicology to the ecological effects of chemicals. Ecotoxicological studies measure the effects of a chemical substance in the environment on indigenous populations of organisms. They provide a mechanism to estimate hazard. Ecotoxicological data are appropriately interpreted with knowledge of the ecosystem where the organisms live. In aquatic ecotox studies, what may be toxic under conditions created in the laboratory, may be more or less toxic in the aquatic environment due to factors present in the aquatic ecosystem which affect bioavailability. Also the chemical itself may be transformed as a result of physical and biological mechanisms, including metabolism. An accurate evaluation of the toxicity of a chemical requires knowledge of these factors.

Sulfonated perfluorochemicals appear to produce a variety of responses in single species tests of aquatic organisms. Different species have varied significantly in their response to the same chemical even when using the same laboratory procedure. In ecotoxicology, environmental concentration often substitutes for knowing the actual amount or dose of a chemical entering an organism, but concentration and dose may not be directly related and their relationship varies from species to species.

Basic environmental toxicity screening data are available for many sulfonated perfluorochemicals (see Table 12), although their quality is variable. In considering the toxicity test results, it is important to note the year of the test. Test protocols typically were developed considering water soluble, stable and well-dispersed compounds. Compounds such as sulfonated perfluorochemicals challenge test protocols due to their insolubility, polymeric, or surface active nature. The older data may reflect these test limitations. Older test protocols are not comparable to recent and current bioassays that follow accepted, standardized test methods (OECD/USEPA).

Almost all previous testing used products which are complex mixtures and not purified perfluorochemicals. In old tests, the sulfonated perfluorochemical product used was likely more variable, with more impurities because manufacturing processes and product purity have significantly improved over time. Several tests were hampered by the insolubility of the perfluorochemical and results are expressed as greater than the measured solubility.

Two sulfonated perfluorochemicals have more toxicity test data than others because of their use as insecticides in ant and roach bait stations. These perfluorochemicals are N-EtFOSA and PFOS Li salt. Toxicity data on these compounds may be found in the disclosures filed by other registrants under the Federal Insecticide, Fungicide and Rodenticide Control Act (FIFRA).

3M has evaluated the reliability of its aquatic toxicity test data base. The numerical descriptor is modeled after the reliability coding used by EPA's Office of Toxic Substances for the AQUIRE (Aquatic Information Retrieval) toxicology data base.

Table 12. Ecotoxicity Testing on Sulfonated Perfluorochemical Products

Pimephales promelas= Fathead minnow
Salmo gairdneri= Rainbow trout
Selenastrum capricornutum= Green algae
Lepomis macrochirus= Bluegill sunfish
Daphnia magna= Water Flea
Microtox=*Photobacterium phosphoreum*

Reliability Codes:
1. Study used published test guidelines or well-documented procedures. Control performance was satisfactory. Toxicant concentration was measured. Test water temperature, pH and dissolved oxygen were measured.
2. Study meets all the criteria for quality testing but has one or more of the following deficiencies:
   A. Nominal test substance concentration; actual concentration not measured.
   B. Test water quality variables not reported or incomplete.
   C. A water accommodated fraction (WAF) was used.
   D. Analytical methodology was questionable.
3. Study does **not** meet the criteria for quality testing. Characterized by one of the following:
   A. Demonstrated weaknesses in experimental procedures.
   B. A static test with unmeasured concentrations was conducted in the presence of precipitate or some undissolved chemical
   C. Insufficient description of methods.
D. Unsatisfactory control mortality.

Product's Principal Fluorochemical	Test Organism	Study Type	Results mg/L	Year	Reliability Code
POSF	*Pimephales promelas*	96 hr LC50	>1000	84	2A
N-MeFOSE alcohol	*Lepomis macrochirus*	96 hr LC50	>solubiliy	79	3B
	Daphnia magna	48 hr LC50	>solubiliy	79	3B
N-EtFOSE alcohol	*S. capricornutum*	14 day EC50	>1800	81	3B
	Pimephales promelas	30 day hatch, growth,survival histopathology	.020	78	2D 2D 2D
		NOEC	.020	78	2D
		LOEC	>.020		
N-EtFOSA	*Daphnia magna*	48 hr EL50	14.5	98	2A,C
		48 hr EL10	7.3	98	2A,C
		48 hr NOEL	5.8	98	2A,C
	Pimephales promelas	96 hr LL50	206	98	2A,C
		96 hr LL10	115	98	2A,C
		96 hr NOEL	130	98	2A,C
	Ceriodaphnia dubia	48 hr EL50	328	98	2A,C
		48 hr EL10	184	98	2A,C
		48 hr NOEL	216	98	2A,C
	Daphnia magna	48 hr EC50	3.2	84	3B
	Pimephales promelas	96 hr LC50	34	84	3B

Product's Principal Fluorochemical	Test Organism	Study Type	Results mg/L	Year	Reliability Code
N-EtFOSEA	*Pimephales promelas*	96 hr LC50	>1000	84	3B
N-EtFOSEMA	*Pimephales promelas*	96 hr LC50	>1000	84	3B
PFOS NH_4 salt	*Pimephales promelas*	96 hr LC50	85	74	2A
	Pimephales promelas	96 hr LC50	100	74	2A
PFOS Li salt	Microtox *P.phosporeum*	30 min EC50	>1000	94	2A
	Daphnia magna	48 hr EC50	210	94	2A
		48 hr NOEC	100	94	2A
	Pimephales promelas	96 hr LC50	19	94	2A
PFOS K salt	Microtox	30 min EC10	45	91	2A
		30 min EC50	>280	91	2A
	Daphnia magna	48 hr EC50	27	84	2A
		28 day NOEC	7	84	2A
	Selenastrum capricornutum	4 day EC50 cell count	82	82	2A
		14 day EC50 cell count	95	82	2A
	Pimephales promelas	30 day NOEC	1	78	2D
		30 day LOEC	1.9	78	2D
		96 hr LC50	38	77	2A
	Lepomis macrochirus	96 hr LC50	68	78	2A
	Salmo gairdneri	96 hr LC50	11	78	2A
	Daphnia magna	48 hr EC50	50	79	2A
	Pimephales promelas	96 hr LC50	29	74	2A
	Pimephales promelas	96 hr LC50	32	73	2A
PFOS DEA salt	*Lepomis macrochirus*	96 hr LC50	31	79	2A
		96 hr NOEL	18	79	2A
perfluoroC10 sulfonic acid, NH_4^+ salt	*Daphnia magna*	48 hr EC50	44	92	2A
	Pimephales promelas	96 hr LC50	4.8	92	2A
	Microtox	30 min EC50	330	92	2A
K salt of carboxylic acid analogue of N-Et-FOSE alcohol	*Pimephales promelas*	96 hr LC50	97	97	2A
		96 hr NOEC	54	97	2A
	Selenastrum capricornutum	96 hr EC50	600	97	2A
		96 hr NOEC	216	97	2A
	Daphnia magna	48 hr EC50	9.1	97	2A
		48 hr NOEC	3.9	97	2A
	Microtox	30 min IC50	270	97	2A
	Pimephales promelas	96 hr LC50	518	81	3B
		96 hr LC50	15	74	3A
N-EtFOSE alcohol ethylene oxide adduct	*Lepomis macrochirus*	96 hr LC50	285	78	2A
	Daphnia magna	48 hr EC50	1.5	78	2A

Table Key

EC50= Median Effective Concentration. It is the concentration of a test substance that causes a 50% effect on a specific characteristic of the test organisms (e.g. immobilization of 50% of the Daphnia, reduction in algal cell growth by 50% as compared to the controls) after a specified exposure period. It is the usual endpoint in a toxicity test with Daphnia and other small organisms where death is hard to determine or in tests where growth is measured.

LC50= Median Lethal Concentration. It is the concentration of a substance that kills 50% of the test organisms exposed to it in a specified time. It is the usual endpoint in an acute toxicity test with fish.

IC50= Median Inhibitory Concentration. It is the concentration of a test substance that inhibits a biological process of a test organism by 50% (e.g. light production, respiration) after a specified exposure period

NOEL= No Observed Effect Level

NOEC= No Observed Effect Concentration

EL=Effective Loading, **LL**=Lethal loading. These are used where the test substance is not completely water soluble. A water accomodated fraction (WAF) is prepared. The test substance is loaded into water at different loadings to prepare each test concentration. The solutions are mixed and the liquid fraction is decanted to use as the test water.

Additional studies are underway on ecotoxicity using established OECD/EPA methods. Initially purified PFOS and EtFOSE alcohol are being tested to determine acute and chronic toxicity to a wide range of species. The results to date are found in Table 13.

Table 13. New Ecotox Studies on PFOS, potassium salt

Parameter	Study Type	Results,
Wastewater Bacteria (OECD 209)	3 hr NOEC 3 hr. EC50 Inhibition @ highest conc (1000 mg/L)	1.0 mg/L >1000 mg/L 39%
Senenastrum capricornutu (green algae)	96 hr NOEC (growth rate) 96 hr ErC10 96 hr. ErC50	48 mg/L 65 (59-69) mg/L 138 (125-149) mg/L
Daphnia magna (freshwater flea)	Acute 48 hr NOEC Acute 48 hr EC10 Acute 48 hr EC50 Acute 48 hr EC90 21 day semi-static life cycle NOEC 21 day semi-static life cycle NOEC	36 mg/L 57 (<12->99) mg/L 66 (36-99) mg/L 69 (<12->99) mg/L *13 mg/L* 26 mg/L
Mysidopsis bahia (marine shrimp)	Acute 96 hr NOEC Acute 96 hr EC50 35 day flow thru life cycle NOEC 35 day flow thru life cycle NOEC	1.2 mg/L 4.0(3.3-5.0) mg/L *0.28 mg/L* *0.6 mg/L*
Freshwater mussel	Acute 96 hr NOEC Acute 96 hr LC50	22 mg/L 65 mg/L
Pimephales promelas (fathead minnow)	Acute 96 hr. NOEC Acute 96 hr LC50 47 day early life-stage toxicity NOEC 47 day early life-stage toxicity LOEC	3.6 mg/L 10 (8.8-12) mg/L *0.33 mg/L* 0.65 mg/L
Oyster Shell Deposition	Acute 96 hr NOEC Acute 96 hr EC50 (Solubility limits precluded EC50) Inhibition @ highest conc (3.3 mg/L)	2.1 mg/L >3.3 mg/L 28%
Avian Dietary Toxicity Testing	Acute Mallard Duck LC50 Acute Mallard Duck, no mortality Acute Mallard Duck NOEC Acute Bobwhite Quail LC50 Acute Bobwhite Quail, no mortality Acute Bobwhite Quail NOEC	*730 (532-1059) mg/kg* *160 mg/kg* *40 mg/kg* *214 (163-260) mg/kg* *80 mg/kg* *80 mg/kg*

Data in italics are from draft reports.

All of the results shown in Table 12 suffer from limitations in the reliability of the data, and there is a clear need for high quality ecotoxicity data using established OECD/EPA methods. Testing on purified PFOS and EtFOSE alcohol is in progress. However, the available new data (Table 13) and the historic data are consistent in that almost all toxicity values for PFOS and related sulfonated perfluorochemicals are greater than 1 mg/L and most are greater than 10 mg/L. An exception is the fathead minnow results reported on Table 12 for N-EtFOSE alcohol where apparently there is no acute toxicity at

or above the water solubility level. The available new data on PFOS itself suggest that it has similar aquatic toxicity to that of other anionic surfactants (9). Few other conclusions can be reliably drawn at this time. For instance, ecological risk assessment typically relies on chronic toxicity values, but there are too few data on this to draw any conclusions.

11.0 Comprehensive Plan to Assess Environmental Exposure

The ongoing activities described in the previous sections of this paper are being carried out as part of a 3M developed comprehensive plan using a combination of 3M resources and outside experts. This plan, summarized in Figure 1, is designed to assess the potential pathways of environmental exposure associated with the manufacture, use and disposal of its sulfonated perfluorochemical products.

11.1 Plan Overview

The plan structure consists of four components:

1. Characterize the properties critical to understanding the fate and transport of sulfonated perfluorochemicals.

2. Estimate the releases of sulfonated perfluorochemicals.

3. Characterize the distribution of sulfonated perfluorochemicals in the environment.

4. Estimate human and ecological exposure to sulfonated perfluorochemicals.

Several individual research projects feed information into each component. The early components provide information needed to complete the later ones. Thus the information base expands when one goes from component 1 to component 4.

This section provides an overview of the plan and its research projects. Specific descriptions of how and why the research projects are being conducted can be found in the preceding sections of this document. The results generated by this plan will be combined with the ecotoxicological studies to develop an assessment of risk. A tentative initiation date for each of the research projects is found in Figure 2. It is expected that the studies will continue over several years.

Figure 1. Diagram of Fluorochemical Assessment Plan.

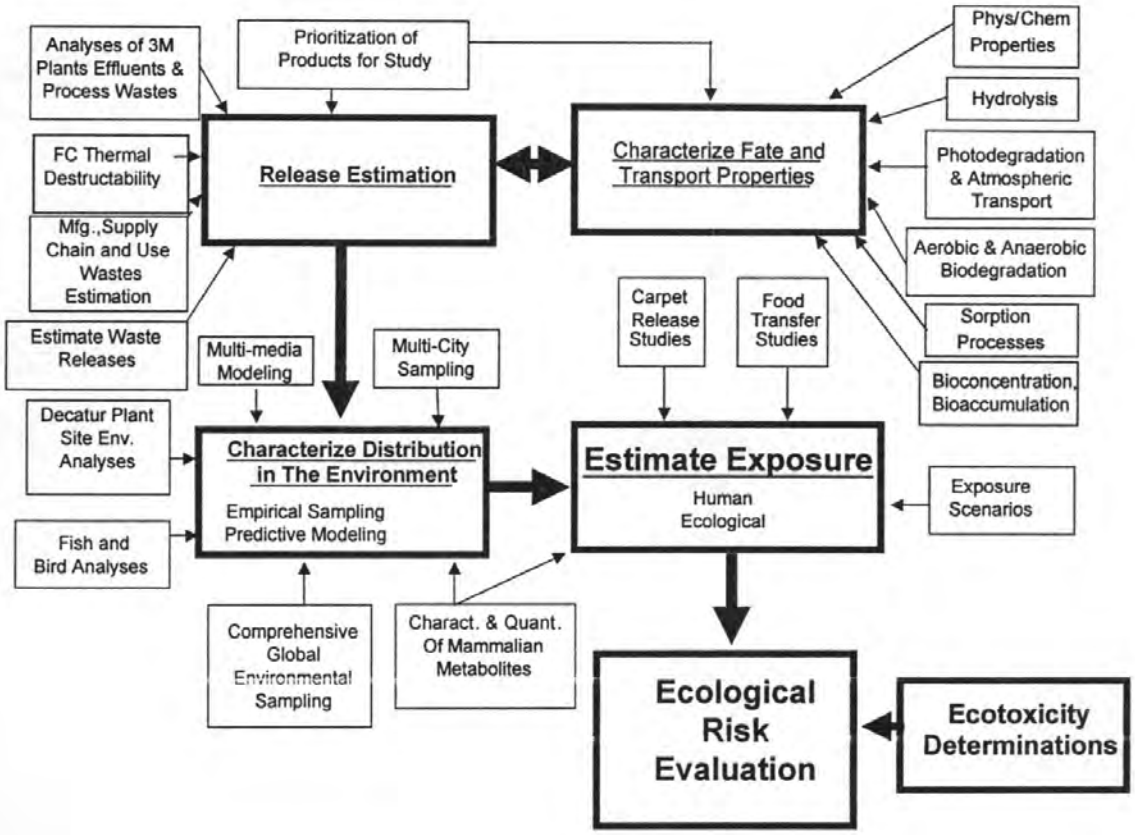


Figure 2. Schedule for FC Exposure Plan Components.

1Q=First Quarter, 2Q= Second Quarter, 3Q=Third Quarter, 4Q=Fourth Quarter

		Initiation Dates
Characterize Fate & Transport Properties	PFOS Phys/Chem Properties	Complete
	EtFOSE alcohol Phys/Chem Properties	1Q 2000
	MeFOSE alcohol Phys/Chem Properties	1Q 2000
	Hydrolysis	1Q 1999
	Photodegradation and Atmospheric Transport	1Q 1999
	Biodegradation (aerobic and anaerobic)	1Q 1999
	Sorption Processes	2Q 1999
	PFOS Bioconcentration	2Q 2000
	EtFOSE alcohol Bioconcentration	4Q 2000
Estimate Releases	3M Plant Effluent & Process Waste Analyses	1Q 1999
	Estimate Mfg, Supply Chain, & Use Waste Streams	Complete
	Estimate Waste Releases	1Q 1999
	FC Thermal Destructability	2Q 1999
Characterize Distribution in Environment	Bird & Fish Analyses	Complete
	U.S.Bird Livers Analyses	Complete
	Biosphere Sampling Pan	1Q 1999
	Multi-media Modeling	1Q 2000
	Multi-cities Study	1Q 1999
Estimate Exposure	Carpet Study	2Q 1999
	Paper and Packaging Studies	1Q 1999
	Exposure Scenarios	2Q 1999
Ecotoxicity Determinations	PFOS Acute Ecotoxicity	1Q 1999
	PFOS Chronic Ecotoxicity	2Q 1999
	EtFOSE alcohol Acute Ecotoxicity	2Q 2000
	EtFOSE alcohol Chronic Ecotoxicity	3Q 2000
	FOSA Acute Ecotoxicity	1Q 2000
	FOSA Chronic Ecotoxicity	2Q 2000

11.2 Component 1: Characterize Fate and Transport Properties

This component was developed in three steps. First, the important fate and transport mechanisms were identified. Next, priorities were set for testing, with the likely degradation products having the highest priority for testing. Finally, methods and laboratories were selected to do the testing. For sulfonated perfluorochemicals not tested, models are being developed that will predict physical and chemical properties. The specific research projects underway are:

1. Physical and chemical properties testing.
2. Hydrolysis testing.
3. Photodegradation and atmospheric transport testing.
4. Anaerobic and aerobic biodegradation testing.
5. Soil and sediment sorption testing.
6. Bioconcentration testing.

11.3 Component 2: Estimate Releases

This component was also developed in several stages. First, product sales data from 1997 were used to identify a study set of products. The study set was based on volume of use and waste streams, mode of release and product chemistry. Next, evaluation efforts of the waste streams generated focused on those study set products sold by 3M in the greatest quantities in the United States. Commercial and residential uses of these products, including transportation, handling and application during the supply chains that lead to product use, were examined. Additionally, the releases likely to result from disposal during these portions of the products' life cycles were also estimated. The estimates included disposal via incineration, landfilling and wastewater treatment.

Waste streams generated at the start of the products' life cycles, i.e. the manufacturing process, were also examined. Better estimates are continuing to be developed of waste streams occurring during the manufacturing process.

11.4 Component 3: Characterize Distribution in the Environment

This component is distinguished by iterative interaction between modeling and field sampling. Models are being used to suggest sampling locations and detection limits. Field sampling is planned to obtain empirical data to validate model output and improve predictions. As new data become available, research projects become more refined and focused. The research projects completed or planned to characterize environmental distribution include:

1. Field sampling of environmental media near the Decatur manufacturing plant.
2. Screening of eagle and albatross plasma and fish tissue from archived samples.
3. Analysis of wild bird livers.
4. Biosphere sampling plan to determine levels in biota of different geographic locations.
5. Development of a multimedia model for predicting distribution.
6. Multi-cities studies in which cities of a similar size are paired, one demonstrating significant manufacturing or commercial uses of sulfonated perfluorochemicals, the other having no identified use of sulfonated perfluorochemicals.

11.5 Component 4: Estimate Exposure

When data from the release and distribution components are available, hypotheses will be developed about important exposure pathways. Iterative sampling and modeling will be used to test these hypotheses and to determine the important exposure pathways to be used in risk assessment. The research projects planned to estimate exposures are:

1. Carpet releases and links to ingestion, inhalation and dermal exposure.
2. Paper and packaging studies and ingestion exposure.
3. Exposure scenarios which combine information from the release, fate, and sampling studies.

12.0 Ecotoxicity Determinations

In conjunction with the studies described in the four component plan described above, 3M is conducting ecotoxicological studies. Ecotoxicological studies are used to estimate hazard. Initial ecotox testing is focusing on PFOS, EtFOSE alcohol, and FOSA. The results of the release, distribution and exposure assessments may provide reasons to test more substances.

The following research projects on ecotoxicity are planned or underway:

1. Aquatic acute toxicity studies: sewage microorganisms, freshwater and marine algae, duckweed, daphnia, mysid shrimp, freshwater mussels, fathead minnows.
2. Terrestrial acute toxicity studies: mallard duck and bobwhite quail dietary exposure studies, earthworm toxicity studies, and green plant growth and uptake studies.
3. Aquatic chronic toxicity studies: oyster shell deposition, daphnia, mysid shrimp, frog embryo development and fish early life stage studies.

4. Terrestrial chronic toxicity studies: mallard duck and bobwhite quail reproduction.

13.0 Ecological Risk Evaluation

Evaluating ecological risk is more complex and more uncertain than assessment of human health risks where a clearer connection can be drawn between dose and response.

As this comprehensive science and exposure assessment program progresses, a framework in which ecological risk can be evaluated will be developed. The evaluation of ecological risks and human risks will both use information about distribution in the environment and exposures generated by this comprehensive exposure plan. Because of the scope and magnitude of the overall program, aspects of the knowledge gained will be compartmentalized into discreet elements to create this framework. For example, as data on ecotoxicological properties, fate and transport mechanisms, and environmental distribution are developed, they will be used to evaluate ecological risk within a certain geographic area or locality. The science data and environmental sampling results will be applied to a very specific area and set of species to evaluate relative risks in that area. Building a number of these compartmental evaluations will result in a much more complete picture of ecological risk. This evaluation will identify additional actions 3M could take to minimize the releases of sulfonated perfluorochemicals.

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THE SCIENCE OF ORGANIC FLUOROCHEMISTRY

3M

February 5, 1999

The Science of Organic Fluorochemistry

Introduction

The science of fluorochemistry begins with fluorine. Fluorine is the most abundant member of the halogen family and is one of the most reactive of all the elements. It is capable of combining with nearly every other element in the periodic table, which is why elemental fluorine is rarely if ever found in nature. The strength of the fluorine bond with other elements also made the discovery of elemental fluorine a difficult task. Elemental fluorine was not isolated until 1886, a relatively late date, as chemical discoveries go. Therefore, the science of fluorochemistry is relatively young. Because of its strong electronegative properties, ionic fluorine will form weak bonds with other electronegative atoms and very strong bonds with electropositive atoms. Ionic metal fluorides are the most common chemical forms of fluorine found in nature, such as fluorspar (CaF_2). Naturally occurring fluorinated hydrocarbon molecules rarely occur in nature because of the energy required to make or break the carbon-fluorine bond in biological systems. However, partially and fully fluorinated organic molecules can be synthesized.

3M Experience in Fluorochemistry

3M Company began its history in fluorochemistry with the licensing of specific intellectual property from Dr. Simons of Penn State University in 1945. Dr. Simons had developed a process, now referred to as Simons Electro-Chemical Fluorination (ECF), to synthesize organofluorine molecules. In this process, organic feedstocks are dispersed in liquid, anhydrous hydrogen fluoride, and an electric current is passed through the solution, causing the hydrogen atoms on the molecule to be replaced with fluorine. The predominant component of the products produced by this process have the same carbon skeletal arrangement as the feedstock used with all of the hydrogen atoms replaced by fluorine. However, fragmentation and rearrangement of the carbon skeleton can also occur and significant amounts of cleaved, branched and cyclic structures may be formed. The degree of fluorination of the organic feedstock is also dependent upon the specific carbon chain length of the feedstock and parameters of the ECF process such as electrical current and the length of time the process is run. It is possible to synthesize fully fluorinated or perfluoroorganic molecules where all of the hydrogen atoms of the hydrocarbon feedstock have been replaced by fluorine atoms. 3M built the first manufacturing pilot scale ECF process in 1949 and has continued to develop and improve the Simons ECF process for the production of fluorochemical products. Currently, 3M has three manufacturing sites in the United States using the ECF process (Cottage Grove, MN, Cordova, IL, and Decatur, AL).

3M Production of Sulfonyl-based Fluorochemicals

3M has produced sulfonyl based fluorochemicals commercially for over 40 years using the ECF process. A basic building block of such products and the highest production volume fluorochemical 3M manufactures is perfluorooctanesulfonyl fluoride (POSF). The starting feedstock for this reaction is 1-octanesulfonyl fluoride. (Reaction 1)

Reaction 1

$$2\ C_8H_{17}SO_2F + 34\ HF \xrightarrow[\text{ECF}]{\text{4.5-7.0 V}} 2\ C_8F_{17}SO_2F + 17\ H_2$$

1-Octanesulfonyl fluoride — Perfluorooctanesulfonyl fluoride (**POSF**)

It is important to understand that perfluorooctane sulfonic acid (**PFOS**) will result from the chemical or enzymatic hydrolysis of POSF. Under appropriate conditions, the perfluorooctane sulfonate anion can form salts with monovalent metallic cations. Current information strongly supports that PFOS or its salts cannot be broken down further chemically. Therefore PFOS is the ultimate degradation product from POSF derived fluorochemicals and will persist in that form.

The electrochemical fluorination process yields about 35%-40% straight chain (normal) POSF, and a mixture of biproducts and waste of unknown and variable composition comprised of the following:

1) higher and lower straight-chain homologs, i.e., n-$CnF_{2n+1}SO_2F$, e.g., $C_6F_{13}SO_2F$, $C_7F_{15}SO_2F$, $C_9F_{19}SO_2F$ which comprise about 7% of the process output

2) branched-chain, perfluoroalkylsulfonyl fluorides with various chain lengths, about 18-20% of the output

3) straight-chain, branched, and cyclic (non-functional) perfluoroalkanes and ethers, which comprise about 20-25% of the output

4) "tars" (high molecular weight fluorochemical byproducts) and other byproducts, including molecular hydrogen, which comprise about 10-15% of the output.

Because of slight differences in process conditions, raw materials, and equipment, the mixture produced by the electrochemical fluorination process varies somewhat from lot-to-lot and from plant-to-plant. The product that results from electrochemical fluorination is thus not a pure chemical but rather a mix of isomers and homologues. The commercialized POSF derived products are a mixture of approximately 70% linear POSF derivatives and 30% branched POSF derived impurities.

During production, byproducts and waste products are formed. The volatile waste products, such as perfluoromethane, have been vented to the atmosphere in the past, but improvements are underway to capture and destroy these releases by thermal oxidation. The tars are incinerated at an in-house, hazardous waste incinerator. The byproducts, many of which are incompletely fluorinated with hydrogen atoms still present, can be recycled back into the ECF process or are partially degraded in stabilization processes, and discharged to controlled, in-house, wastewater treatment systems. The treatment sludge is either landfilled or land-incorporated. Some of the non-POSF byproducts are recovered and sold for secondary uses.

POSF is itself a commercially viable product, but is primarily an important intermediate in the synthesis of substances used in many other 3M fluorochemical products. The majority is used to produce functionally derivatized fluorochemicals and high molecular weight polymeric products. Table 1 identifies some fluorochemicals, their acronyms, chemical name, and formulas. To a lesser extent, some homologues of POSF, [$C_nF_{(2n+1)}SO_2F$ where n=other than 8], principally perfluorohexanesulfonyl fluoride, are also intermediates in the formation of other 3M products. PFOS is also a commercialized product for a variety of specific applications.

Using POSF as a basic building block, unique chemistries can be created by derivatizing POSF through the sulfonyl fluoride moiety of the molecule using conventional hydrocarbon reactions. Chart 1 outlines the general classes of fluorinated materials made by 3M. The major intermediates are represented by the trunk of the "tree". POSF is reacted with methyl or ethyl amine to produce either N-methyl or N-ethylperfluorooctanesulfonamide (FOSA). FOSA is subsequently reacted with ethylene carbonate to form either N-methyl or N-ethylperfluorooctanesulfonamidoethanol (FOSE).

Chart 1

POSF Fluorochemical Reaction Tree

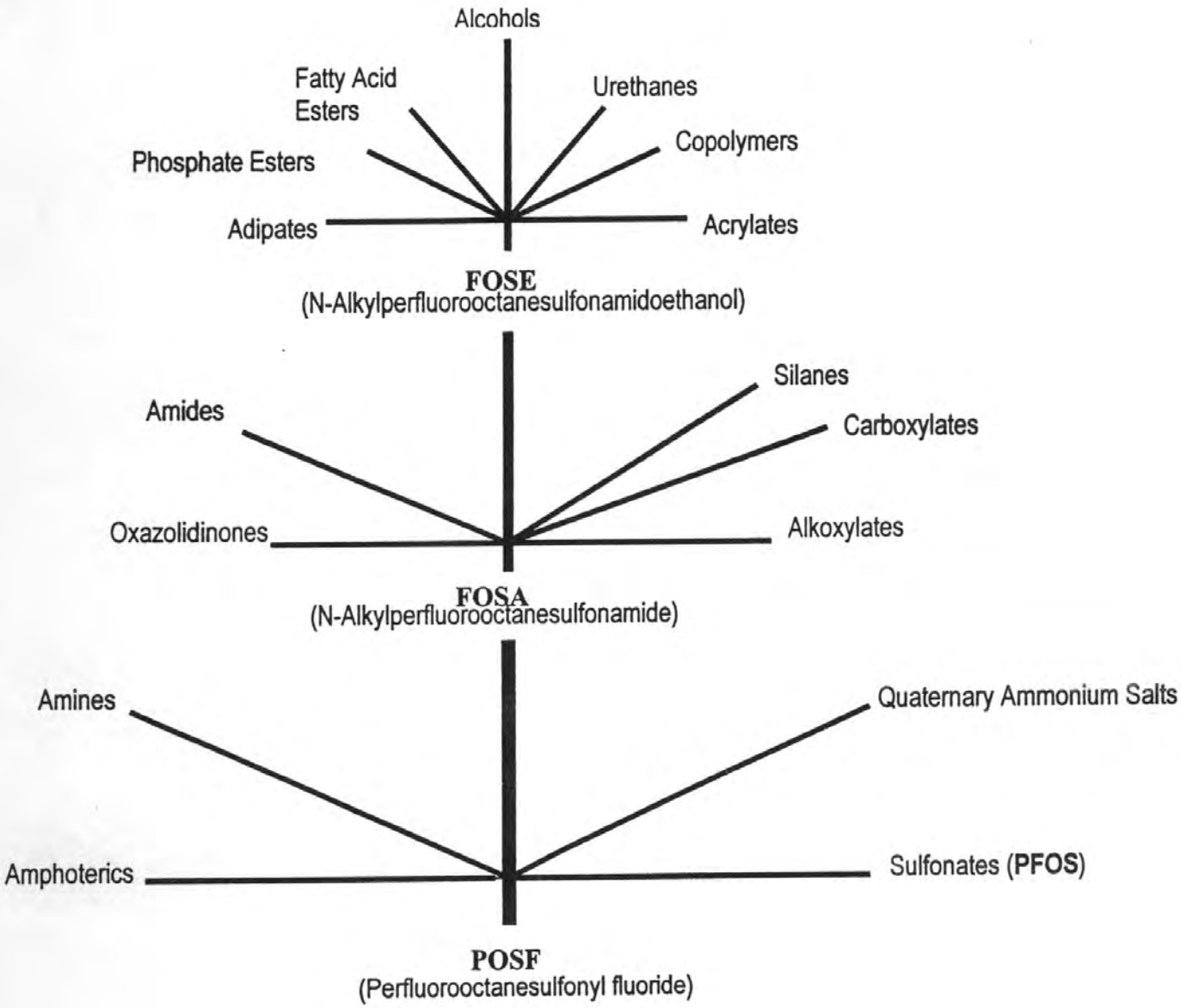


Table 1. Fluorochemical Glossary

Designation	Molecular Formula	Technical Name (CAS Name)
POSF	$C_8F_{17}SO_2F$	Perfluorooctanesulfonyl fluoride (1-Octanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
PFOS	$C_8F_{17}SO_3^-$	Perfluorooctanesulfonate (1-Octanesulfonic acid anion, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
PFOSH	$C_8F_{17}SO_3H$	Perfluorooctanesulfonic acid (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
PFOS.NH_4 salt	$C_8F_{17}SO_3NH_4$	Ammonium perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, ammonium salt)
PFOS.DEA salt	$C_8F_{17}SO_3NH(CH_2CH_2OH)_2$	Perfluorooctanesulfonate, diethanolamine salt (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1))
PFOS.K salt	$C_8F_{17}SO_3K$	Potassium perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt)
PFOS.Li salt	$C_8F_{17}SO_3Li$	Lithium perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, lithium salt)
FOSA	$C_8F_{17}SO_2NH_2$	Perfluorooctanesulfonamide (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
N-EtFOSGE	$C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$	N-perfluorooctylsulfonyl-N-ethylglycinate (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, anion)
N-EtFOSA	$C_8F_{17}SO_2NHCH_2CH_3$	N-Ethylperfluorooctanesulfonamide (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
N-MeFOSA	$C_8F_{17}SO_2NHCH_3$	N-Methylperfluorooctanesulfonamide (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-methyl-)
N-EtFOSE	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$	N-Ethylperfluorooctanesulfonamidoethanol (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-)
N-MeFOSE	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$	N-Methylperfluorooctanesulfonamidoethanol (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-N-methyl-)
N-EtFOSEA	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OCOCH=CH2$	N-Ethylperfluorooctanesulfonamidoethyl acrylate (2-Propenoic acid, 2-[ethyl[(heptadecafluorooctyl)sulfonyl]amino]ethyl ester)
N-EtFOSEMA	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OCOC(CH_3)=CH2$	N-Ethylperfluorooctanesulfonamidoethyl methacrylate (2-Propenoic acid, 2-methyl-, 2-[ethyl[(heptadecafluorooctyl)sulfonyl]amino]ethyl ester)
N-MeFOSEA	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OCOCH=CH2$	N-Methylperfluorooctanesulfonamidoethyl acrylate (2-Propenoic acid, 2-[[(heptadecafluorooctyl)sulfonyl]methylamino]ethyl ester)

The secondary reactions producing all of these derivatives are single or sequential batch processes that do not necessarily produce pure products. There may be varying amounts of fluorochemical residuals (unreacted or partially reacted starting materials or intermediates, such as FOSA or FOSE) that are carried forward to the final product. Typically, these residuals are present at a concentration of 1% or less. Such processes are under a continuous improvement plan to reduce or eliminate the presence of unnecessary residuals in the production of commercializable product. It is important to understand that the non-fluorochemical moieties added to the sulfoniyl fluoride group of POSF can also be removed through a variety of degradation processes (chemical, environmental, and metabolic). Again, the terminal product of such degradation will be PFOS.

Physical-Chemical Properties

Fluorinated organics are less well described in the scientific literature than organic molecules bearing other halogens, i.e. bromine, and chlorine, which have been more thoroughly investigated by many researchers in published reports. To understand fluorinated organic properties, it is necessary to describe in more detail the properties of fluorine. Fluorine has several characteristics which differ from the other halogens and contribute to the unusual properties of fluorochemicals.

Fluorine has a van der Waals radius of 1.35 Å, more comparable to that of oxygen and smaller than other halogens, and is isosterically similar to a hydroxyl group. Fluorine has the highest electronegativity (4.0 – Pauling scale) of all the halogens, and the highest in the periodic table. This confers a strong polarity to the carbon-fluorine bond. The carbon-fluorine bond is one of the strongest in nature (~110 kcal/mol). See Table 2 and 3. This very strong, high energy bond contributes to the stability of fluorochemicals. Such stability may also lead to the persistence of certain fluorochemicals. That stability confers a variety of unique properties to fluorocarbons as described in Table 4.

TABLE 2
Fluorocarbon Structure Considerations

Structure	Bond Length A°	Atomic Van Der Waals' Radius A°	Bond Strength Kcal/Mole
H_3C-H	1.11	1.20	101
H_3C-F	1.385	1.35	107
H_3C-Cl	1.78	1.80	81
H_3C-Br	1.93	1.95	67
H_3C-I	2.13	2.15	55

TABLE 3
Effect of Increasing Fluorination

Structure	Bond Length A°	Bond Strength Kcal/Mole
H_3C-F	1.385	107.0
H_2FC-F	1.358	109.6
HF_2C-F	1.332	114.6
F_3C-F	1.317	116.0

TABLE 4
Stability of Fluorocarbons

Chemical
- Unaffected by any normal reagent
- React with alkali metals at high temperatures

Thermal and Oxidative
- Stable in air at high temperatures
- Non-flammable

Electrical
- High electric strength
- Low dielectric constant

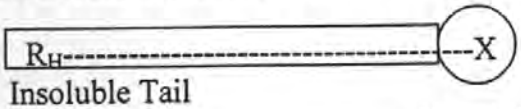
The high ionization potential of fluorine (401.8 kcal/mole) and its low polarizability leads to weak inter- and intramolecular interactions. This is demonstrated in the low boiling points of fluorochemicals relative to molecular weight, and their extremely low surface tension and low refractive index. Table 5 compares the physical properties of a perfluoroalkane with its hydrocarbon analog to demonstrate the effect of low polarizability.

TABLE 5
Physical Properties
(Effect of Low Polarizability)

	C_8F_{18}	C_8H_{18}
Low Boiling Point:		
MW	438	114
Bp (°C)	97	125
Low Heat of Vaporization		
G-Cal/Gram	20	86.8
Low Refractive Index		
N_D20	1.280	1.3975
Low Surface Tension		
Dynes/cm	15.0	21.8

The partitioning behavior of perfluoroalkanes is also unique. Some perfluoroalkanes, when mixed with hydrocarbons and water, form three immiscible phases, demonstrating that perfluorinated chains are both oleophobic and hydrophobic. A charged moiety, such as carboxylic acid, sulfonic acid, phosphate or a quaternary ammonium group, when attached to the perfluorinated chain, makes the molecule more water soluble because of the hydrophilic nature of these charged moieties. Therefore, such functionalized fluorochemicals can have surfactant properties.

A conventional hydrocarbon surfactant generally may be represented as:

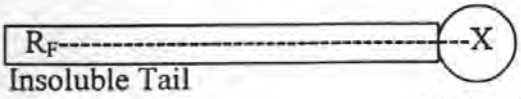


Insoluble Tail

Hydrophilic Group

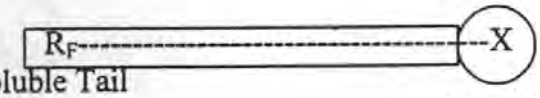
Where R_H....represents the hydrocarbon "tail" and ····X represents a solubilizing group.

In a similar fashion, fluorochemical surfactants can typically be described by the following chemical structure:



Where the R_F.... portion is the stable fluorocarbon tail, ···X represents a solubilizing group. It is this unique fluorochemical "tail", modified in length and structure to meet end use needs, which provides the exceptional resistance to thermal and chemical attack characteristic of the fluorochemical. This fluorochemical portion of each is basically responsible for its capability to dramatically reduce surface tension, as well as being the major difference between these materials and conventional surfactants.

The solubilizing group, ···X is commonly water soluble, but can be designed to be oil soluble for use in nonaqueous systems.



The nature of the oleophilic group varies among the fluorochemical surfactants. By altering it, fluorochemical surfactants have been prepared which are extremely surface active in a number of environments, including many systems which would degrade hydrocarbon or silicone surfactants.

Physical properties available on 3M fluorochemical products are principally those parameters needed for quality control use and material handling. It is important to remember that these physiochemical properties have been obtained from products that are not highly refined, and that may have more than one fluorochemical component. Some products may have nonfluorochemical components which contribute to the determination of the values. One observes a wide range in values for physiochemical parameters among low molecular weight, POSF based, fluorochemicals. Typically these low molecular weight chemistries tend to have higher water solubility and lower vapor pressure than polymeric products containing them. In addition to being intermediates in the formation of products, some of these low molecular weight fluorochemicals are also likely intermediates in the degradation of polymeric compounds.

3M Sulfonyl-based Fluorochemical Products

The 3M product lines that use POSF-based fluorochemicals are summarized below. In some cases, 3M manufactures the final commercialized product. In other cases, 3M sells a fluorochemical which another company incorporates into their final product. (Product lines using fluorochemicals which contain no sulfonyl groups are not listed.)

Surface Treatments

Fabric/Upholstery Protector (High molecular weight [MW] polymers)
Carpet Protector (High MW polymers)
Leather Protector (High MW Polymers)
Paper and Packaging Protector (High MW phosphate esters or high MW polymers)

Surfactants (Low MW chemical substances)
- Specialty Surfactants
- Household Additives
- Electroplating and Etching Bath Surfactants
- Coatings and Coating Additives
- Chemical Intermediates
- Carpet Spot Cleaners
- Fire Extinguishing Foam Concentrates
- Mining and Oil Surfactants

Other Uses
- Insecticides (Low MW chemical substances)

Some of the POSF derived chemistries are relatively low molecular weight (< 500 daltons). These fluorochemicals can be intermediates that 3M or our customers use in making other finished products. Such fluorochemical intermediates can be covalently bound to a variety of polymeric hydrocarbon backbones to make products with unique performance characteristics. The majority of 3M fluorochemicals produced for commercialization are used in such polymeric form for treatment of surfaces and materials. For example, fluorochemical containing polymers (urethane and acrylate) plus fluorochemical adipates can provide soil, stain, and water resistance to personal apparel and home furnishings. Such protective products function through the fluorocarbon moiety on the polymer lowering the surface energy of the material to which they are applied.

The 3M paper protectors can be divided into two general classes of chemistries. One class is based on phosphate esters of N-EtFOSE. The other class is a N-MeFOSEA-acrylate copolymer. Applied to paper, the fluorocarbon moiety in these products has the previously described effect of lowering the surface energy of the individual paper fibers. This lowered surface energy greatly contributes to the holdout of low surface energy liquids such as greases and oils.

As previously described, the POSF-derived fluorochemical products have surfactant properties. Such fluorochemical surfactants differ greatly from conventional hydrocarbon and silicone surfactants. In most systems they are far more efficient in reducing surface tension to levels that are unreachable with these other types. In some aqueous systems, surface tensions as low as 15 to 16 dynes/cm can be attained. The fluorochemical surfactants normally produce these extremely low values at concentrations as low as 100 parts per million, or less. Equally important is the fact that certain of these fluorochemical surface active agents are stable and effective in many extremely hostile environments, including strongly acidic, strongly alkaline and even strongly oxidizing systems. Table 6 summarizes the features of fluorochemical surfactants.

Table 6
Features of Fluorochemical Surfactants

SURFACE ACTIVITY

AQUEOUS SYSTEMS

Some of these surfactants can lower surface tension to less than 16 dynes/cm and function at low concentrations. They are effective in dramatically reducing surface tension in a wide variety of aqueous media, including acidic and basic systems.

NON AQUEOUS SYSTEMS

Fluorochemical Surfactants have been developed which uniquely reduce surface tensions of many organic media to about 20 dynes/cm, including solvents such as esters, alcohols and ethers and resin systems including epoxies, polyesters, urethanes and acrylics.

WETTING

Reduced surface tensions result in the ability to improve the wetting of a variety of materals, including such hard to wet surfaces as plastics and oily metals.

BETTER SPREADING

Low surface tension in combination with low interfacial tension affects spontaneous spreading of a liquid over various surfaces. This is important in reducing pinholes, craters, and edge crawling of coatings applied to unclean surfaces.

REDUCED WATER SPOTTING

Because of reduced droplet formation, the need for distilled or deionized water in rinsing operations may be eliminated.

SMALLER GAS BUBBLES

These smaller gas bubbles produced at the surface of metal during chemical etching will have less tendency to adhere, grow and cause surface imperfections.

SMALLER DROP FORMATION

Smaller drops are desired in fine aerosol mists.

BETTER LIQUID PENETRATION

The force required to cause liquids to move through small pore spaces can be greatly reduced.

IMPROVED FILM UNIFORMITY

Smoother, more even films are produced from polishes, finishes and coatings.

LEVELING

Emulsion coatings applied to difficult to wet surfaces can show greatly improved leveling with the addition of small quantities of these materials.

FOAMING

Stable foams can be produced in hostile media such as chromic acid or sodium hydroxide, where conventional surface active agents would be destroyed.

EMULSIFICATION

While generally not effective as emulsifiers in water-organic systems, these materials can be quite efficient emulsifiers in specialty applications, where fluorinated materials comprise either the continuous or the dispersed phase.

STABILITY

Chemical

Some of these surface active agents are stable in such rigorous environments as hot chromic acid, anhydrous hydrazine, hot concentrated sulfuric acid, hot concentrated hydrofluoric acid and hot concentrated sodium hydroxide solutions.

Thermal

While all of these materials have very good stability at moderate temperatures, a few can withstand temperatures in excess of 300°F in air.

LOW CONCENTRATION

These materials are normally effective at extremely low concentrations, and often are utilized at concentrations of 100 parts per million active solids or less.

Many applications involve more than just air-liquid interfaces where surface tension alone might be important. More often liquid-liquid or solid-liquid systems are encountered. In these cases, interfacial tension, as well as surface tension, plays a significant role in the wetting or leveling process. Quite often in these cases, a combination of a suitable hydrocarbon surfactant can produce a degree of wetting which cannot be accomplished by either type alone. Normally, in such a combination, it is the fluorochemical surfactant which reduces the surface tension, while the hydrocarbon material aids in the reduction of the interfacial tension. The net result can be a system that easily wets and spreads on otherwise hard to wet surfaces.

Another unique physical characteristic of fluorochemicals is their ability to form tough, yet resilient foams. Such foams have been formulated to resist the action of high temperature or aggressive chemicals and vapors. These formulations have found commercial application in suppressing flammable liquid, chemical and organic fires or toxic and obnoxious vapors and odors.

Perfluorooctane Sulfonate: Current Summary of Human Sera, Health and Toxicology Data

3M

January 21, 1999

TABLE OF CONTENTS

January 21, 1999

Executive Summary

3M has prepared this document to summarize the data related to the biological effects of perfluorooctane sulfonate (PFOS). It also presents current thinking on human health risk related to PFOS and includes information about future study plans. 3M Medical Department scientists and physicians, in consultation with outside experts, are the authors.

PFOS has been found at tens of parts per billion levels in serum samples of nonoccupationally exposed employees, in commercially available human serum and in pooled samples from multiple blood banks. PFOS is an eight-carbon molecule that is perfluorinated except for the sulfonate group on the terminal carbon. 3M has manufactured PFOS and molecules that may be metabolic precursors to it since 1948. Routes of exposure to PFOS or precursor molecules are not well understood at this time.

PFOS is an example of an "organic" fluorine molecule. Human serum has been known to contain organic fluorine molecules for over 30 years. The primary constituent of this organic fluorine fraction was tentatively identified as another molecule (perfluorooctanoate) in 1976. Current analysis of stored sera samples from a variety of sources are more consistent with PFOS being a major fraction of this organic fluorine. Improved analytic techniques allowing a relatively rapid analysis at low levels of detection make the current analyses possible. These analytic techniques were first available for use in medical surveillance of exposed workers in 1992. Detection limits have been lowered to allow the more recent analysis of serum from those without occupational exposure.

Medical surveillance has been done among 3M employees occupationally exposed to PFOS precursors for over 20 years. To date, no adverse health effect associated with PFOS exposure has been found in these employees. This conclusion applies at serum levels up to 6 parts per million, about 100 times higher than levels seen in the general population. PFOS has a long residence time in the human body. In three retirees, the half-life in human sera ranges from 1100 to 1500 days. A mortality study at the U.S. plant

primarily involved with production of PFOS related materials has found no significantly elevated standardized mortality ratios (SMR's).

Toxicology studies show that PFOS is well absorbed orally and distributes primarily in the serum and liver. It does not appear to be further metabolized. Some enterohepatic circulation of PFOS occurs, based on the observation of increased excretion in rats given cholestyramine. Elimination from the body is slow and occurs via both urine and feces.

Mutagenicity testing is negative in five salmonella species. It is not genotoxic in a mouse bone marrow micronucleus assay. The acute LD50 in rats is 250 mg/kg (moderately toxic). It does not produce dermal or ocular toxicity.

Subchronic studies have been done in rats and primates. PFOS causes liver enzyme elevations and hepatic vacuolization in rats, and hepatocellular hypertrophy at higher doses. Higher doses also cause other GI toxicity, hematological abnormalities, weight loss, convulsions, tremors and death. Monkeys show anorexia, emesis, diarrhea, hypoactivity and at higher doses prostration, convulsions and death. Atrophy of exocrine cells in salivary glands and the pancreas, and lipid depletion in the adrenals is found at high doses in the monkey.

The serum levels at which these compound related effects occurred in these early rhesus monkey studies are unknown. In a recently completed rangefinder study in cynomolgus monkeys the first observed biological effect was a decrease in serum cholesterol, first observed at a serum level of 72 ppm in one of the two monkeys in the high dose group. Using the relationship between cumulative dose and serum level found in this study, it can be estimate that significant toxicity occurred at 700 to 800 ppm in the early rhesus monkey studies, and death at 1100 ppm and above. More complete quantitative absorption, distribution and excretion data for PFOS is being obtained.

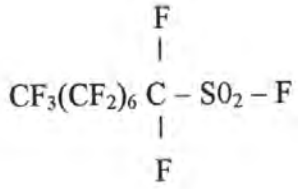
Available information therefore suggests that no identifiable health risk to humans would be expected to occur at the PFOS levels found in blood bank or commercial serum samples.

Extensive further research, which includes epidemiological and laboratory studies, is planned or underway. The purpose of this research is to explore the potential for chronic and reproductive effects, understand toxic mechanisms and obtain a better understanding of absorption, distribution, metabolism and excretion. The plan is to make as much use as possible of observational data in exposed workers and to establish no effect levels in both rats and primates for endpoints of importance.

I. INTRODUCTION

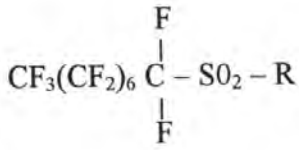
Evidence that organic compounds containing the element fluorine covalently bonded to carbon (organic fluorine compounds, OF) can be found in human sera has been available for 30 years. Although all of the specific compounds contributing to the total amount of OF present are not identified, it now appears that a compound called perfluorooctane sulfonate constitutes a significant fraction. Recent data provide evidence that PFOS is present at tens of parts-per-billion (ppb) levels in serum samples from the general population, averaging 30 ppb in blood bank samples from diverse locations in the U.S. Single digit parts per million (ppm) levels (approximately 100 times greater) are found in individuals occupationally exposed to PFOS and its precursors, averaging 2.0 ppm among participating employees at the primary U.S. manufacturing location for these compounds.

3M produces perfluorinated molecules by mixing anhydrous HF and hydrocarbon feed stock in an electrochemical cell (electrochemical fluorination). Perfluorooctane sulfonyl fluoride (POSF) is the cell product from which a group of products is developed:



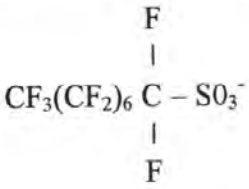
POSF

Other moieties ("R") are added to the sulfur, which leads to the creation of materials that may be polymerized or esterified. The vast majority of POSF produced is used in this way.



POSF derived molecule

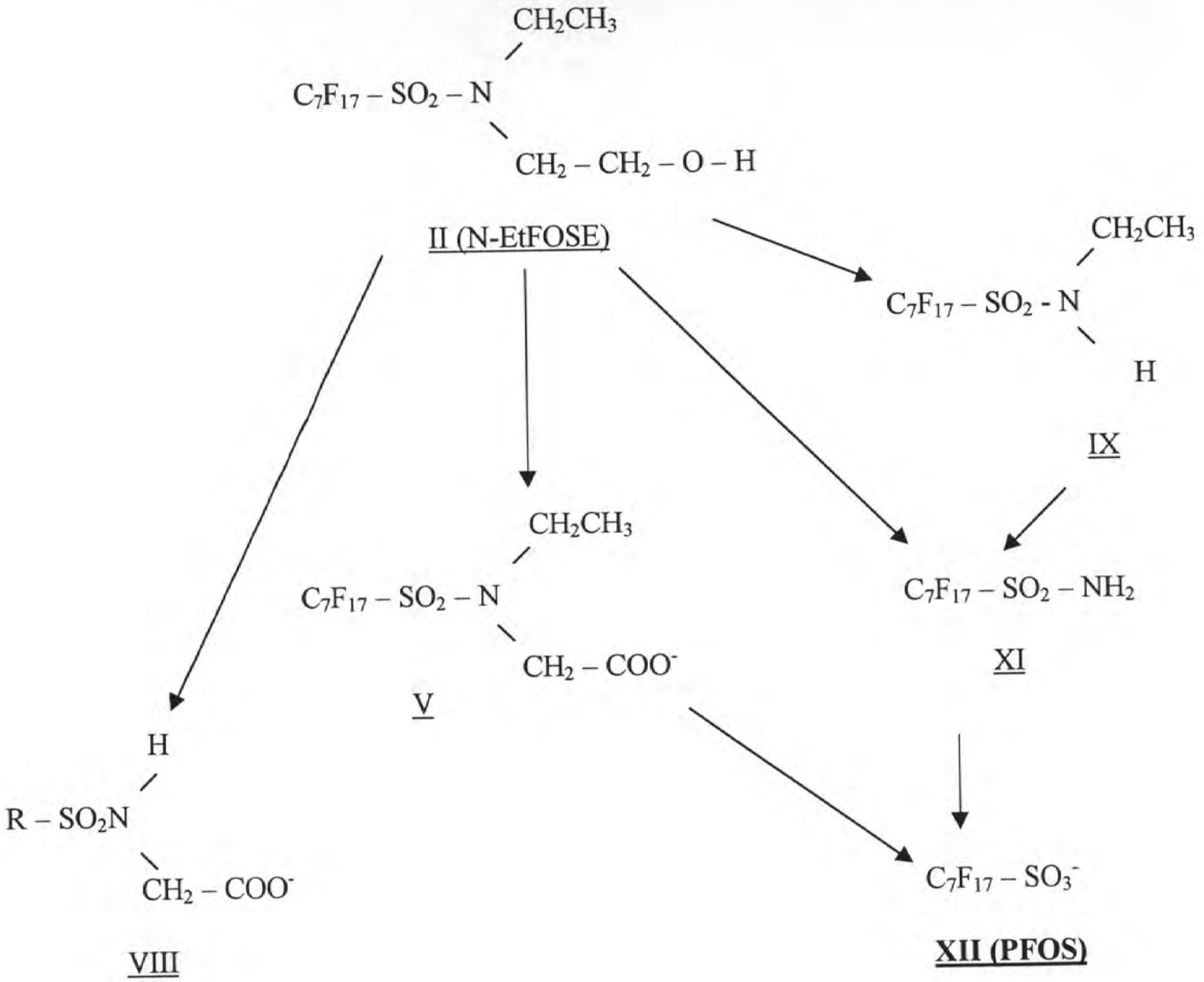
PFOS may result from the loss of the "R" moiety through metabolic processes. Current evidence would indicate that PFOS is not further metabolized. Some PFOS is produced and sold directly into industrial applications as a surfactant. This, however, amounts to only a small fraction of total POSF production.



PFOS

Most POSF that is produced is used in 2-(N-ethylperfluoroctanesulfonamido)-ethyl alcohol (N-Et-FOSE) and 2-(N-methylperfluoroctanesulfonamido)-ethyl alcohol (N-Me-FOSE) based products. Figure I.1 shows the chemical structure of N-Et-FOSE and metabolites that have been found in rat serum. All except compound VIII have been verified to metabolize further to PFOS. (Missing Roman numerals represent hypothesized intermediates not shown in this figure.)

Figure I.1 N-EtFOSE(*) Metabolites Identified in Rat Serum



In vitro studies in both rat and human hepatocytes lead to identification of the same compounds and hypothesized intermediates. Compound VIII has been detected in some samples of pooled human sera. A single dose absorption, distribution, metabolism and excretion study of N-Et-FOSE in cynomolgus monkeys is through the in life phase and tissue analysis is pending. N-Et-FOSE is esterified to produce larger molecules that are used on paper and packaging for oil and water repellency.

It is presumed that N-Me-FOSE, in which a methyl group replaces the ethyl group on the nitrogen, has a similar metabolism. N-Me-FOSE becomes part of very large molecules that act as protective chemicals on fabrics, leather and rugs.

Outside of the occupational setting, routes of human exposure to PFOS or its metabolic precursors are not understood, but are the subject of intense study. Exposure could occur from environmental releases of PFOS or its precursors at the Decatur, Alabama and Antwerp, Belgium manufacturing sites. It could occur from the environmental or biological degradation of products to PFOS or molecules metabolized to PFOS. Products also contain small amounts (generally less than a few percent) of residuals, such as N-Et-FOSE and other molecules found in Figure I.1, which are known or suspected metabolic precursors to PFOS. These residuals represent a source of PFOS that would not require environmental or biological degradation of large molecules. Downstream industrial users of POSF based products are also potential sources of environmental releases of PFOS or its precursors. The relative contribution of these various sources to population exposure is currently unknown.

Another surfactant is known to be found in the sera of employees and was reported in general population sera samples in 1976 (Taves). This is perfluorooctanoic acid, or PFOA:

$CF_3(CF_2)_6COOH$

PFOA is also made by electrochemical fluorination. It is mentioned here because following Taves' report in 1976 it was presumed to be a significant fraction of the total organic fluorine found in the sera samples analyzed by Taves and others. This report was a stimulus for investigation and subsequent medical surveillance of employees in fluorochemical production, including those producing POSF based materials. It should be recognized, however, that PFOA is a different and unrelated compound. It does not metabolize to or become PFOS. It is likely that PFOA was misidentified as a major fraction of organic fluorine in the 1976 Taves paper. The evidence for this is discussed in Section II.

The purpose of this report is to describe the data on PFOS levels in human sera, and to discuss the potential for those levels to affect health based on current scientific knowledge. A review of current findings and historical information on PFOS levels in sera is presented in Section II. This is followed in Section III with a description of 3M's epidemiology and medical monitoring database obtained from studies of its workers in plants in the United States and Belgium. The animal and other laboratory toxicology data available on PFOS are presented in Section IV. Section V offers a preliminary evaluation of the serum findings in light of the available health effects data.

3M is actively developing further human health and toxicological information. Section VI outlines the current research agenda.

II. HISTORICAL REVIEW AND CURRENT FINDINGS OF FLUOROCHEMICALS IN HUMAN SERA

The data on fluorochemicals in human sera is presented in this section. The presence of organic forms of fluorine in human serum was observed 30 years ago, and PFOA was thought to account for most of this fraction. 3M finds little current evidence to support this view. Evidence is presented below that PFOS is more likely to be consistently found in sera. Based on the limited data provided by historical samples, there is no evidence of significant change in PFOS concentration in serum samples taken over the last two to three decades.

The advancement of analytical chemistry technology has had a significant influence on our knowledge of fluorocarbons in human sera. The techniques developed and used by researchers in the 1960's and 1970's were time intensive, requiring hours for a single analysis. The methods were also nonspecific, measuring organic fluorine (fluorine covalently bonded to carbon) rather than specific molecules. The development of a rapid analytic technique in the late 1970's decreased analytic time to under an hour, allowing large scale medical surveillance of production employees at higher detection limits (about 0.5 parts per million organic fluorine) that were adequate for the levels found in occupationally exposed individuals. The advancement of chromatographic/mass spectroscopy technology enabled rapid analysis of specific fluorochemicals from small volumes of sera in the early 1990's. This technology was first used in medical surveillance in 1992. Detection limits for PFOS were lowered to 50 parts per billion by 1997. The first report to 3M of PFOS in commercially available pooled sera occurred in late summer of 1997, prompting more research into the technique, and confirming its validity over a period of several months.

Since older published data described organic fluorine content rather than a specific molecule, it is useful to understand the relationship between PFOS levels currently

observed and organic fluorine content. Fluorine is 65% of the molecular weight of PFOS. The contribution of a PFOS value to organic fluorine, in ppb, will therefore be [0.65 x (PFOS value in ppb)]. Conversely, if the measured organic fluorine is entirely PFOS, the value of PFOS in ppb will be [1.54 x (OF level in ppb)].

This section presents 1) a brief summary of the historical information regarding organic fluorine in human sera, 2) data from 3M employees involved in fluorochemical production, 3) data from a small group of non-occupationally exposed 3M employees, 4) data from commercially available human pooled serum samples, 5) data from pooled sera from 18 regional blood banks and 6) data from current analysis of stored serum samples.

Historical Finding of the Organic Form of Fluorine in Blood

Taves (1968a) described two forms of fluorine in serum, one that was exchangeable with radioactive fluorine-18 and one that was not. Pothapragada et. al. (1971) also described two forms, ionic and nonionic. Taves (1968b) showed that the non-exchangeable fluorine was bound to albumin. This finding, along with results of extraction and precipitation and the need for ashing to release this form of fluorine, led to the conclusion that the non-exchangeable or nonionic fluorine was "organic", i.e. covalently bound to carbon (Taves et. al., 1976). Using NMR spectroscopy, these authors tentatively identified a component of the organic fluorine as perfluorooctanoic acid (PFOA). There was some variation in the observed spectra from an authentic sample of PFOA, however, leading the authors to suggest that branching, or the presence of a sulfonate, was possible.

A number of studies over the past 25 years reported levels of organic fluorine in human blood serum. Table II.1 presents the study author, level measured, population studied and methods of analysis. The variety of methods used for determination of fluorine suggests that some caution be used in interpreting results. All reported means

were in the tens of part per billion levels. The average of reported values from United States sources is 37.6 ppb.

Table II.1.
Historical Findings of Serum Organic Fluorine Levels

Year	Author	OF* (ppb)	N	Method**	Source
1972	Guy	30	65	ash	US
1975	Venkateswarlu	36	2	O bomb	US
1976	Guy, Taves	25	106	ash	US
1978	Belisle	20	9	O bomb	US
1979	Singer	45	264	ash	US
1980	Paez	85	pooled	ash	Argentina
1980	Ubel	45	4	mod O bomb	US
1981	Belisle	11	8	O bomb	China
1989	Yamamota	32	11	LOPA	Japan

* Organic fluorine, specific identities not provided.
** Varied methods were used to measure organic fluorine. See papers for details.

Occupationally Exposed Employees

3M has produced PFOA (the ammonium salt) by electrochemical fluorination since the early 1950's. It is a surfactant used in fluoropolymer production. The company began medical monitoring of employees involved in PFOA production in 1976, by measuring serum levels of organic fluorine (OF) and performing medical assessments. Employee monitoring was expanded significantly in 1980 following the development of a more rapid test for organic fluorine. Measured serum levels of OF in these employees averaged less than 10 parts per million (ppm).

As noted earlier, PFOS is a surfactant used as a wetting and foaming agent in industrial and commercial processes. Certain fluorochemicals that may transform metabolically to PFOS have been produced since the early 1950's by electrochemical fluorination. Since 1980 this has occurred at primarily one location in the United States (Decatur, Alabama). This 3M site consists of a fluorochemical plant and a film plant that are physically separate entities. Chemical plant employees have been offered a medical monitoring program that includes standard medical testing as well as

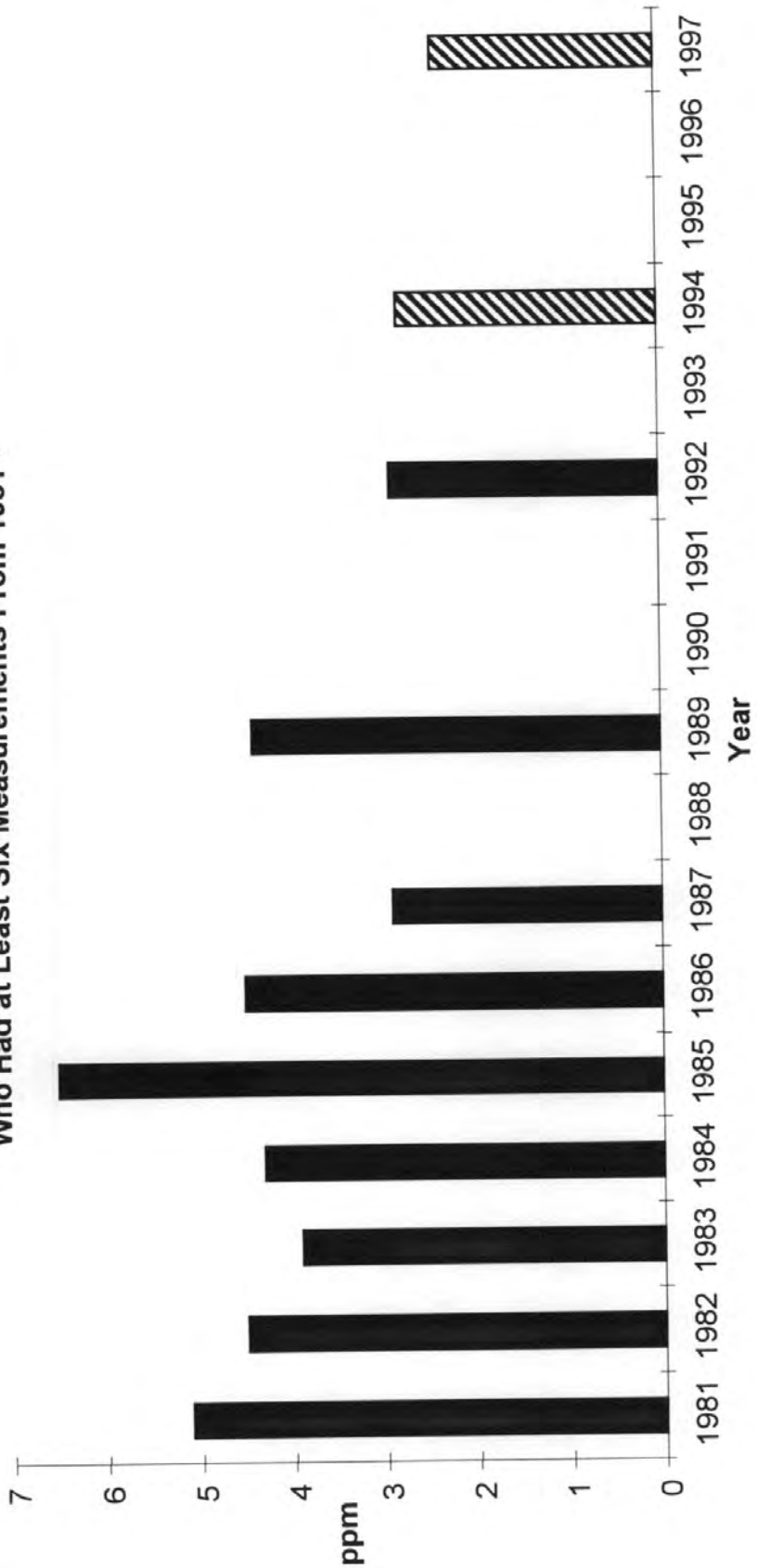
measurement of serum levels of OF. Among employees with six or more measurements, OF levels averaged 2.9 to 6.5 ppm from 1981 to 1992[1] (Figure II.1). With the introduction of high performance liquid chromatography-mass spectrometry, serum PFOS was measured in 1994 and 1997 (see also Figure II.1). In these two years, mean PFOS levels were 2.44 ppm (range 0.25 – 12.83 ppm) and 1.96 ppm (range 0.10 – 9.93 ppm), respectively.

Another 3M plant in the United States where PFOS has been measured in employees' serum is Cottage Grove, Minnesota. Some PFOS is manufactured at this plant. In 1997, the mean serum level of PFOS among 74 Cottage Grove fluorochemical production employees was 0.82 ppm (range 0.05 - 6.25 ppm). Outside the United States, 3M manufactures PFOS related materials at its Antwerp, Belgium plant. PFOS levels were measured in Antwerp employees in 1995 (mean = 1.9 ppm, range 0.0 - 9.9 ppm) and in 1997 (mean 1.5 ppm, range 0.1 - 4.8) ppm.

The cross-sectional stratified analysis presented in section III, examining the relationship between PFOS sera level and various clinical chemistry and hormone parameters, was conducted at the Decatur, Alabama and Antwerp, Belgium plants. The Cottage Grove facility was not included because little PFOS related product is manufactured there. It is also the primary site for PFOA production.

[1] We are aware of one occasion in 1979 where the serum of 5 Decatur employees was measured for PFOS by electron capture gas chromatograph and microwave plasma detection methods [Central Analytical Laboratory, 1979]. Total serum organic fluorine levels for these five employees were 10.1, 5.7, 9.4, 11.8 and 4.1 ppm. The percent of PFOS found was 60%, 70%, 80%, 55% and 65% of the total serum organic fluorine levels, respectively.

Figure II.1.
Mean Levels (ppm) of Total Serum Organic Fluorine (solid black or Perfluorooctane Sulfonate (striped bars) in Decatur Who Had at Least Six Measurements From 1981 -



Non-Occupationally Exposed 3M Employees

A total of 31 3M employees were tested for PFOS in their serum in 1998 (Table II.2). All were corporate staff or division managers. None had worked in fluorochemical production or in fluorochemical research and development. Samples were from five females and 26 males. Employees ranged in age from 37 to 62 years. All employees had measurable PFOS in their blood serum (mean = 47 ppb; range = 28 to 96 ppb). Age was significantly associated with increased serum PFOS and accounted for 24% of the variance in PFOS levels. There was no gender-related difference if age was considered. Only four employees had PFOA measured above the detection limit of 10 ppb. The average of these four PFOA measurements was 12.5 ppb. Twelve employees were re-tested eight weeks later to check for reliability of the analytical method. The findings suggested reliability (R^2 = .94) in the range of quantification (Figure A1 in the Appendix).

Table II.2.
Summary of Mean and Range of PFOS (ppb) Levels in Current and Historical Human Populations,
All Data Analyzed in February - April, 1998

Populations	Description of Sample	Mean* (ppb)	Range* (ppb)
A. Current Populations (blood collected in 1998)			
1. Non-occupationally exposed 3M employees	31 individuals	47	28-96
2. Commercial pooled serum samples			
Intergen Laboratory	3 samples each with ≥ 100 donors	44	43-44
Sigma Laboratory	3 samples each with ≥ 100 donors	33	26-45
3. Pooled serum from 21 separate U.S. blood banks (see Figure 1 for more detail)	3 to 6 pooled samples per location with 5 to 10 donors per sample	30	9 - 56
B. Historical Samples (chronological order)			
1. Korean War era U.S. military recruits, 1948 to 1951	10 pooled samples with 10 donors per sample	N.D.**	N.D.
2. Swedish samples, 1957	10 individual samples	2	N.D. - 2
3. Michigan Breast Cancer Study, 1969-1971	5 individual samples	33	N.D. - 59
4. Swedish samples, 1971	10 individual samples	1	N.D. - 1
5. MRFIT pooled calibration samples, 1976 (Multiple Risk Factor Intervention Trial)	6 pooled samples with unknown number of donors per sample	31	14 - 56
6. MRFIT pooled calibration samples, 1980	3 pooled samples with unknown number of donors per sample	23	14 - 41
7. China samples (Linxian, rural province), 1984	6 individual samples	N.D.	N.D.
8. MRFIT individual samples, 1985	3 individual samples	31	N.D. - 44
9. China samples (Shandong, rural province), 1994	6 individual samples	N.D.	N.D.

* Rounded to nearest ppb
** Not detected

Commercial Pooled Serum Samples

Six pooled sera samples, obtained from two commercial sources (Intergen and Sigma), were tested in 1998. No information was available about the donor pool, such as age, sex or geographical location. Samples from Intergen came from donor pools consisting of approximately 500 individuals. Three Intergen pools showed PFOS levels of 43, 44 and 44 ppb (Table 2A.2). Pools from another source, Sigma, were from an unknown number of donors. The pools from which the samples were drawn were 50 liters, suggesting a minimum of 200 donors. The three Sigma pools contained PFOS at levels of 26, 28 and 45 ppb.

Blood Bank Pooled Sera

Eighteen blood banks from various geographic areas across the continental United States and Alaska each donated three to six pooled samples that had from 5 to 10 donors per pool (Table II.3). Altogether there were 68 total pools, representing 340 to 680 individual donors. All pools contained detectable levels of PFOS in tests performed in 1998. The range found in the pooled serum was 9 to 56 ppb of PFOS (see table on next page). The location means ranged from 14 ppb in Santa Barbara, California to 52 ppb in Greenville, South Carolina. PFOA levels were detected in 20 (about one-third) of the pooled samples but quantifiable in only two samples (12 and 22 ppb).

The blood bank samples from the regional blood banks are not a statistically valid sample of the U.S. population, but do provide, to date, the best estimate of mean PFOS sera levels. The overall mean was 29.7 ppb of PFOS. If one wishes to make a comparison with the organic fluorine levels in the published literature, this level of PFOS would contribute 19.3 ppb to the total serum organic fluorine level (0.65 x 29.7). An approximate mean of historic organic fluorine measurements from published literature is 37.6 ppb. If this were all PFOS, a PFOS sera level would have been 57.9 ppb (1.54 x 37.6).

Table II.3 **PFOS (ppb) Levels from 18 Blood Banks**

City	# of Pooled Samples	Range	Average
Anchorage, AK	4	19-31	24
Billings, MT	3	18-26	23
Cheyenne, WY	3	13-35	23
Corpus Christi, TX	3	26-32	29
Davenport, IA	3	28-40	32
East Orange, CA	3	20-27	24
Grand Rapids, MI	4	18-31	26
Greenville, SC	3	47-56	52
Kansas City, MO	3	24-35	30
Lafayette, LA	3	39-50	46
Las Vegas, NV	3	25-28	23
Meridian, MS	3	27-56	39
Minneapolis, MN	6	41-54	46
Newark, DE	5	21-32	24
Omaha, NE	5	9-27	17
Santa Barbara, CA	3	13-16	14
Santa Rosa, CA	3	23-26	24
Scottsdale, AZ	3	28-47	37

Overall mean = 29.7ppb

Historical Samples

Nine sets of historical samples have been analyzed for PFOS in 1998. These are summarized in chronological order below.

Korean War era U.S. military recruits, 1948 to 1951. Ten pooled samples consisting of ten individual samples each were measured. All were below 1 ppb, the limits of detection.

Swedish samples, 1957. Ten individual samples ranged from below limit of detection to 4.1 ppb. Two were below detection limit (1 ppb). The mean was 1.98 ppb.

Michigan Breast Cancer Study, 1969 - 1971. Five individual samples ranged from 11.8 to 59.4 ppb. The mean was 33.4 ppb.

Swedish samples, 1971. Ten individual samples ranged from below detection limit (1 ppb) to 2.8 ppb. Three were below detection limit. The mean was 1.13 ppb.

MRFIT pooled calibration samples, 1976. MRFIT (Multiple Risk Factor Intervention Trial) was a cardiovascular risk reduction program conducted in the 1970's and early 1980's. The number of donors per pool is unknown. Six pooled calibration samples ranged from 13.7 to 55.5 ppb. The mean was 30.9 ppb.

MRFIT pooled calibration samples, 1980. Three pooled calibration samples ranged from 13.8 to 40.5 ppb. The number of donors per pool is unknown. The mean was 25.5 ppb.

China samples, 1984. Six individual samples from Linxian province in rural China were all below limits of detection or quantitation. The samples were from an NCI study on nutrition and cancer prevention.

MRFIT individual samples, 1985. Three individual samples from participants in the MRFIT study were obtained. One was below limits of quantitation (5 ppb), the other two were 43.3 and 43.9. Assuming the LOQ for the low sample, the mean was 30.7.

China samples, 1994. Six individual samples from Shandong province in rural China were all below limits of detection or quantitation. The samples were from an NCI study on nutrition and cancer prevention.

In addition to the PFOS measurements described above, PFOA was also analyzed in these historical samples. PFOA was not found in any historical sample at a detection limit of 10 ppb.

Comment

Organic fluorine has been noted in human serum since the late 1960's. We have now identified PFOS as a part of this organic fluorine fraction. PFOS-related materials were not produced commercially prior to 1948, and only in small quantities for several years thereafter. Therefore, it is not surprising that samples from 1948 to 1951 show undetectable levels. There was clearly an increase 20 years later; however the very limited data shows no further upward trend despite steadily increasing production volumes since this time. Due to limited knowledge on distribution of this chemical in the body it is uncertain that body burden is adequately reflected by serum levels. Information on distribution and kinetics is needed to shed further light. The available data on this topic will be presented in the toxicology section. It would also be of interest to measure total organic fluorine and PFOS in the same sample to determine how much PFOS currently contributes to the total organic fluorine content.

PFOA may have been misidentified as a major component of organic fluorine in 1976 (Taves, 1976). Although detectable in some samples, neither historic nor current samples confirm this as a major fraction, except in occupationally exposed employees.

III. SUMMARY OF MEDICAL SURVEILLANCE AND EPIDEMIOLOGY STUDIES

Epidemiologic Investigation of Clinical Chemistries, Hematology and Hormones in Relation to Serum PFOS Levels in Male Fluorochemical Production Employees

Medical surveillance has been routinely performed on 3M fluorochemical production workers (in Decatur, Alabama and Antwerp, Belgium) with potential exposure to PFOS and/or to perfluorinated precursors that may metabolically degrade to PFOS. A recent study (Olsen et al., 1998) provided an analysis of hematology, clinical chemistries and hormonal parameters in relation to serum PFOS as determined by high performance liquid chromatography mass spectrometry methods. These relationships were assessed in fluorochemical production employees from two time periods, 1995 (N = 178) and 1997 (N = 149). [2] In 1995, for Antwerp and Decatur, the mean serum PFOS levels were 1.93 and 2.44 ppm, respectively. In 1997, the mean serum PFOS levels were 1.48 and 1.96 ppm, respectively.

Descriptive simple and stratified analyses, Pearson correlation coefficients, analysis of variance and multivariable regression were used to evaluate for possible associations between PFOS and each hematological and clinical chemistry test and hormonal assay. Age, body mass index, current alcohol consumption (drinks per day) and cigarette use (cigarettes smoked per day) were potential confounding factors that were considered in the analyses.

Four categorizations of serum PFOS levels were assessed in relation to the response variables: 0 - < 1 ppm; 1 - < 3 ppm; 3 - < 6 ppm; and ≥ 6 ppm (Table III.1). (Note: other PFOS categorizations were used with comparable findings). In 1995, mean serum PFOS levels by category were 0.49 ppm, 1.82 ppm, 4.12 ppm and 8.17 ppm,

[2] Hematocrit, hemoglobin, red blood cells, white blood cells and platelet count, alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, blood urea nitrogen, creatinine, glucose, cholesterol, low density lipoproteins, high density lipoproteins and triglycerides, cortisol, dehydroepiandrosterone sulfate, estradiol, follicle stimulating

respectively. In 1997, mean serum PFOS levels by category were 0.52 ppm, 1.78 ppm, 3.87 ppm and 7.20 ppm, respectively. For both years, 95 percent of the employees' serum PFOS levels were below 6 ppm. The two plant populations differed by age, body mass index and alcohol consumption which resulted in differences, as expected, in several clinical chemistry parameters (Table III.2). When analyzed in aggregate, the two plant populations showed no consistent significant associations for both years between the clinical chemistries and hematology parameters and the employees' serum PFOS levels (Figure A1, Appendix). Total bilirubin levels appeared to trend downwards but upon further analysis this was restricted to Decatur employees and the values were all within the reference range.

Multivariable regression models were fitted with PFOS analyzed as a continuous variable using linear as well as non-linear transformations in order to maximize the possibility of finding associations between PFOS and the parameters of interest adjusting for potential confounders. No consistent associations were observed by plant and/or by year. In 1995, hormone values were also obtained from a sub-sample of employees with the higher PFOS measurements. After adjusting for age and body mass index, no significant associations were observed between hormones and serum PFOS levels.

The findings from this study suggest that, among these Antwerp and Decatur male fluorochemical production employees, significant hematological, clinical chemistry and hormonal abnormalities were not associated with serum PFOS levels up to 6 ppm. It was not possible to derive inferences from the few employees with serum PFOS levels $\geq$ 6 ppm. Limitations of this study included its cross-sectional design, the voluntary participation rates and the few subjects with levels $\geq$ 6 ppm.

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hormone, 17-alpha hydroxyprogesterone, luteinizing hormone, prolactin, sex hormone binding globulin, free testosterone, bound testosterone, and thyroid stimulating hormone.

Table III.1

Distribution of Employees by Year, Location and PFOS Exposure Level (ppm)

	1995 Data						1997 Data					
	All Employees		Antwerp		Decatur		All Employees		Antwerp		Decatur	
PFOS	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
0 - < 1 ppm	45	25	34	39	11	12	60	40	31	48	29	35
1 - < 3 ppm	91	51	32	36	59	66	63	43	25	38	38	45
3 - < 6 ppm	35	20	19	22	16	18	21	14	9	14	12	14
>= 6 ppm	7	4	3	3	4	4	5	3	0	0	5	6
	178	(100)	88	(100)	90	(100)	149	(100)	65	(100)	84	(100)

Table III.2

Mean Values of PFOS, Demographic, Serum Chemistry and Hematologic Parameters for Antwerp and Decatur, 1995 and 1997 Examinations

	1995 Data		1997 Data	
Variable	Antwerp	Decatur	Antwerp	Decatur
PFOS (ppm)	1.93	2.44	1.48	1.96
Age	37***	45	33***	44
BMI	23.9***	29.2	23.5***	30.0
Cigarettes	4.7*	7.9	5.5	6.6
Alcohol	1.3***	0.2	1.1***	0.1
Alk Phosphatase	75***	97	70***	87
GGT	41	48	26*	36
AST	26*	29	27	26
ALT	44	47	31	34
Total bilirubin	0.86***	0.58	0.80***	0.58
Direct bilirubin	0.22	0.21	0.15***	0.12
BUN	17.0***	14.8	14.9	14.1
Creatinine	0.9***	1.1	0.9**	1.0
Glucose	81***	92	81***	98
Cholesterol	214	218	206	215
LDL	138	136	134	137
HDL	54***	43	50***	42
Triglycerides	115***	187	111***	192

Table III.2 (Continued)

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
Hematocrit	47***	46	46*	45
Hemoglobin	15.4	15.2	15.4	15.3
RBC	4.9	5.0	5.1	5.1
MCH	31.4***	30.7	30.5	30.4
MCHC	32.9***	33.4	33.3***	33.7
MVC	95.7***	91.8	91.6*	90.0
WBC	6.4***	7.5	6.5	6.4
Platelets	224	229	237*	217

* $p < .05$; ** $p < .01$; ***$p < .001$ (Antwerp compared to Decatur)

Mortality Study of Employees at the 3M Plant in Decatur, Alabama

A retrospective cohort mortality study of 1,957 employees who worked at least one year at the 3M plant in Decatur, Alabama was conducted by epidemiologists at the University of Minnesota School of Public Health (Mandel and Johnson, 1995). The purpose of this study was to determine whether the mortality experience of these employees was significantly different from that which would be expected in a comparable population. The cohort was followed from March 1, 1961 through December 31, 1991. Table A2 (Appendix) provides a description of the final employment and vital status for the male (n = 1,639) and female (n = 318) employees. These totals include both the Decatur chemical and film plant employees. The two plants are physically distinct entities (approximately 300 yards apart) located at the Decatur site. The vital status of 99.7% of the cohort was identified. The male and female employees contributed 33,108 and 4,807 person-years experience, respectively (Table A3, Appendix). The majority of the male employees were hired between 1965 and 1974 (Table A4, Appendix) whereas the majority of the female employees were hired between 1975 and 1979 (Table A5, Appendix). Altogether there were 74 deaths (70 males and 4 females). Among males, SMRs were below the null value for all major causes of death regardless of the comparison population used to calculate the expected values (Table III.3). Data analyses were also restricted to examining the mortality experience of employees who ever (n = 1,050) and only (n = 485) worked in the Decatur chemical plant (Tables A6 and A7, Appendix). Neither analysis resulted in significantly ($p < .05$) elevated SMRs. There were no analyses conducted by duration of employment within the chemical plant. The data were also restricted to employees who were ever (n = 1,116) and only (n = 547) employed at the Decatur film plant (data not shown). None of these analyses resulted in significantly elevated SMRs. There were four deaths among female employees (SMR = 62.6; 95% CI 17.1 – 160.4). Of these four deaths, three were from external causes (SMR = 213.4; 05% CI 44.0 - 623.6). By examining the death certificates for the specific causes of death and circumstances surrounding the

deaths, it was determined that the excess mortality among females due to external causes was not work-related. The only recommendation by the University of Minnesota investigators was that the mortality experience of Decatur plant employees be updated in 1998 which would extend the vital status search, via National Death Index records, through 1996.

In addition to the Decatur mortality study, the University of Minnesota investigators have also followed the mortality experience of a cohort of employees associated with fluorochemical production at the 3M Cottage Grove (Gilliland and Mandel, 1993). Unlike the Decatur plant, the greatest potential for exposure at Cottage Grove is to PFOA (perfluorooctanoic acid) although the plant does produce PFOS. The total cohort consisted of 2,788 male and 749 female workers employed between 1947 and 1983. There was no significantly increased cause-specific SMR for either men or women. In a proportional hazard regression model, ten years of employment in jobs associated with the chemical division was associated with a 3.3-fold increase (95% CI 1.02 – 10.60) in prostate cancer mortality compared to no employment in the chemical division. The authors suggested that this finding may be biologically plausible with exposure to PFOA as animal toxicology and human data had associated PFOA with reproductive hormone changes (Gilliland, 1992). However, the authors urged caution in the interpretation of this result due to the nonspecificity of the exposure index as well as the few subjects (n = 4) considered 'exposed.' Subsequent research on the Cottage Grove male workforce involved with PFOA production has provided reasonable assurance that there are no significant hormonal changes associated with PFOA at the serum levels measured (Olsen et al., 1998).

Current and proposed human health and exposure research initiatives are found in Section VI.

Table III.3. Selected Cause - Specific SMRs for Men
by Comparison Population (n = 1,639)

Cause of Death	Observed Deaths	SMR (95% Confidence Interval) U.S.	Alabama	AL Regional Counties
All Causes of Death	70	62.9 (49.0, 79.5)	54.8 (42.7, 69.3)	52.0 (40.5, 65.7)
All Malignant Neoplasms	15	68.4 (38.3, 112.8)	60.9 (34.1, 100.4)	59.9 (33.5, 98.8)
Cerebrovascular Disease	1	33.5 (0.8, 186.8)	27.8 (0.7, 155.2)	26.5 (0.7, 147.5)
All Heart Disease	17	59.1 (34.4, 94.7)	53.2 (31.0, 85.1)	49.1 (28.6, 78.6)
Respiratory Disease	0	--- (0.0, 91.2)	--- (0.0, 80.2)	--- (0.0, 73.9)
External Causes	25	74.5 (48.2, 110.0)	59.0 (38.2, 87.1)	55.0 (35.6, 81.3)

IV. SUMMARY OF TOXICOLOGY DATA ON PFOS

Introduction

This section summarizes the existing database with respect to the potential mammalian toxicity of PFOS. Toxicology data are valuable in identifying potential health effects and provide a basis for risk characterization. The use of this information in risk characterization will be described in Section V.

With few exceptions, past toxicology studies either did not characterize blood and tissue concentrations associated with exposure or were not completely specific in characterizing blood and tissue concentrations. For these past studies, blood and tissue concentrations can only be estimated based on limited pharmacokinetic data and results from more recent studies, most of which have been designed to include a pharmacokinetic component to obtain highly sensitive and specific identification of PFOS in blood and tissues. Also, the extent of potential interspecies and intraspecies variability in pharmacokinetic handling of PFOS is not presently well defined. Comparison of serum concentrations of PFOS from toxicity studies to measured human serum concentrations may be an interesting and convenient comparison; however, body burden and/or target organ tissue concentration may be more meaningful in characterizing risk. The available body of animal toxicity data cannot be related quantitatively and without uncertainty and speculation to the concentrations of PFOS in human blood reported in Section II and the results of epidemiologic investigation as reported in Section III.

Despite the current limitations in the ability to characterize risk based on the relationship of blood and tissue PFOS concentrations from toxicity studies to human blood concentrations of PFOS, the existing toxicology database on PFOS does provide valuable insight into potential health hazards and forms a foundation for risk characterization.

It should be mentioned that the specific sources of PFOS identified in the serum of non-occupationally exposed humans are currently unknown. The PFOS molecule itself may not be the primary source. PFOS in the serum of non-occupationally exposed humans may derive from metabolism of higher molecular weight molecules present in industrial, commercial and consumer products or manufacturing by-products.

The studies described in the following discussion reflect the toxic responses resulting from direct dosing with PFOS. The studies that are summarized in this section were performed with the potassium salt of PFOS unless otherwise annotated. Studies on related compounds are considered outside the scope of this discussion and will only be mentioned when they provide valuable insight into the toxicity of PFOS. Some data will be discussed from studies which are currently in progress or incomplete. Tabular summaries of rodent repeated-dose toxicity studies, primate repeated-dose toxicity summaries and developmental and reproduction studies will be found at the end of this section (Table IV.9, IV.10, and IV.11, respectively). In addition to these summary tables, Tables IV.12 presents the results of rodent and primate 90-day sub-chronic studies organized by increasing nominal mg/kg dose level. A list of studies initiated in 1998 can be found at the end of this section in Table IV.8 and summary descriptions of these studies can be found in the Appendix.

Pharmacokinetics of PFOS

The absorption, tissue distribution, potential metabolism and excretion of PFOS has been studied most extensively in rats by both radiolabel and direct quantitation. Limited data relating oral dose to serum and liver concentrations of PFOS in the cynomolgus monkey is available from a recent 28-day rangefinder capsule dosing study. In addition, serum PFOS concentrations in three retired male 3M chemical workers have been followed in an attempt to estimate an elimination rate constant for the human.

In general, PFOS:

- is well absorbed from the digestive system;
- has some limited potential for dermal absorption;
- distributes predominantly to the blood and liver, with liver concentrations being several times higher than serum concentrations;
- appears to have a serum half-life shorter than the elimination half-life after a single dose;
- appears to have extended elimination half lives in rats, monkeys and humans;
- shows slight but consistent sex differences in serum and liver concentrations on repeated dosing in the rat but not the cynomolgus monkey;
- appears to undergo enterohepatic recirculation;
- does not appear to be metabolized, including conjugation reactions.

Absorption: At least 95% of a single oral dose averaging 4.2 mg/kg [^{14}C]PFOS administered to two groups (24 hour and 48 hour sacrifice) of 3 male Charles River CD rats (248-315 g, mean = 285 g) was absorbed within 24 hours (Johnson and Ober, 1979). The radiochemical purity of the [^{14}C]PFOS used in this and the other radiolabel studies listed below was >99% (Johnson and Behr, 1979).

PFOS was applied to skin that had been clipped free of hair as a suspension in water at 5,000 mg/kg covering 40% of the total body surface area of ten male and ten female rabbits. An impervious plastic sheet occluded the skin for 24 hours and was then removed. Animals were maintained and observed for a 28-day period. Blood samples were obtained on days 1, 7, 14, and 28. Analysis for total blood fluoride was performed on the day one and day 28 samples from a single male and single female. Total serum fluoride values for the male were 10.3 ppm for day one and 130 ppm for day 28. The respective values for the female were 0.9 ppm and 128 ppm. Although this study indicated some dermal absorption, it is limited in that the values from only

two animals were measured, and only from the day one and day 28 samples (O'Malley and Ebbens, 1980).

Three male and three female albino rabbits per dose group were exposed dermally under occlusion for 24-hours to an aqueous suspension of PFOS (0.06 %) at doses equal to 0, 0.003, 0.06, and 0.3 mg PFOS / kg and held for 28 days (Glaza, 1995). Liver samples taken at term were analyzed for total organic fluorine. No quantifiable organic fluorine could be detected in the 28-day livers by combustion or by electrospray mass spectroscopy (Johnson, 1995).

Distribution: By 89 days after a single iv dose of PFOS-^{14}C (mean dose, 4.2 mg/kg) six Charles River CD male rats (initial body weights 262-303 g, mean = 288 g) excreted a mean of 30.2% of the total carbon-14 via urine. Mean cumulative fecal excretion was 12.6%. At 89 days, mean tissue concentration of total carbon-14 expressed as µg PFOS-^{14}C equivalents/g were: liver, 20.6; plasma, 2.2; kidney, 1.1; lung, 1.1; spleen, 0.5; and bone marrow, 0.5. Lower concentrations ($\leq$0.5) were measured in adrenals, skin, testes, muscle, fat and eye. No radioactivity (<0.05) was detected in brain. The carbon-14 in liver and plasma represents 25 and 3 percent of the dose, respectively (Johnson etal, 1979).

Serum PFOS concentrations were measured throughout a 28-day oral rangefinder study in which male and female cynomolgus monkeys weighing 2.1 to 2.4 kg were given capsules containing PFOS at either 0.0 mg/kg/day (two males and two females), 0.02 mg PFOS/kg/day (three males and three females), or 2.0 mg/kg/day (one male and one female) (Thomford, PJ, 1998). The monkeys dosed with PFOS (0.02 and 2.0 mg/kg/day) demonstrated an extremely linear (r-squared > 0.99) increase in serum concentration throughout the exposure period (28 days). There was no apparent sex difference and the individual slopes of the cumulative PFOS dose versus serum PFOS concentration curve appeared to be virtually identical between the monkeys in the two dose groups. The average slope of the curve in the 0.02 mg/kg/day group (n = 6) was

5.22 ± 0.74 ppm PFOS in serum per mg/kg cumulative dose, and for the two monkeys in the 2.0 mg/kg/day dose group, the average was 5.40 ± 0.61 ppm PFOS in serum per mg/kg PFOS cumulative dose. At the end of the 28-day dosing period, serum concentration in the 0.02 mg/kg/day dose group reached approximately 3 ppm and in the 2.0 mg/kg/day dose group, serum concentrations reached approximately 300 ppm.

This data suggests a volume of distribution of 0.19 L/kg for continuous dosing over a dose range of two orders of magnitude for the male and female cynomolgus monkey. This is calculated as follows:

One (1) mg/kg results in 5.3 ppm serum PFOS;

This is equivalent to 5.3 mg/L of serum (mg/L = ppm weight/volume);

One (1) mg/kg given to 2.2 kg monkey results in a total dose of 2.2 mg;

V_d (L) is calculated as 2.2 mg / 5.3 mg/L = 0.41 L;

Normalizing this for body weight gives 0.41 L / 2.2 kg = 0.19 L / kg = V_d (L / kg);

V_d for a 60 kg woman is estimated to be 0.2 L / kg x 60 kg = 12 L = V_d (L).

Metabolism: Preliminary data from analysis of urine, feces and tissues of rats as well as the inherent stability of perfluorinated anions suggest that PFOS is not metabolized (Johnson et al., 1984). Exposure of primary human and rat hepatocytes to PFOS did not result in further metabolism (Gordon, 1998). Analysis by LC/MS of serum and liver samples from studies currently in progress have not revealed any evidence of metabolism.

Excretion: In the previously mentioned study (Johnson etal, 1979) single intravenous doses (mean 4.2 mg/kg) of [^{14}C]PFOS in 0.9% NaCl were administered to male rats. By 89 days after dosing, 30.2% of the administered ^{14}C had been excreted in the urine and 12.6% had been excreted in the feces.

Whole body elimination in the male rat appeared to be biphasic. Initial redistribution from the plasma yielded a plasma elimination half-life of ^{14}C of 7.5 days following single oral administration of [^{14}C]PFOS (mean dose 4.2 mg/kg) to male rats (Johnson and Ober, 1979). In the aforementioned intravenous study, elimination of only 42.8 % of the dose through urine and feces after 89 days indicates that the half-life of elimination from the body is > 89 days in the male rat.

Serum PFOS levels in three retired male 3M chemical workers have been followed for five and one-half years and suggest a mean serum elimination half-life of 1,428 days. Over that time period that these serum samples were taken and analyzed, the analytical method changed from thermo-spray mass spectrometry to electro-spray mass spectrometry, and the analytical laboratories changed. These changes should not have affected the values reported to any appreciable extent. Since urine and feces have not been followed, it is difficult to ascertain if this represents a true elimination half-life from the body; however, this is the closest value for elimination half-life applicable to humans in existence. The actual data from these three retired chemical workers is presented in Table IV.1.

Table IV.1 Serum Elimination of PFOS in Retired 3M Chemical Workers.

	Serum [PFOS] in mg/L (ppm)					
	Retiree 1		Retiree 2		Retiree 3	
	3/4/92	5.8	3/18/92	1.6	3/18/92	4.2
	9/20/93	6.0	11/15/93	1.4	11/29/93	3.3
	3/1/94	4.0	5/5/95	0.7	5/4/95	1.7
	6/19/95	3.0	11/18/97	0.7	10/23/97	1.4
	12/5/97	2.6				
Serum Elim. Constant (λ)	- 0.0132/month		- 0.0136/month		- 0.0176/month	
Serum Elim. $T_{1/2}$	52.5 mo/1575 d		50.9 mo/1527 d		39.4 mo/1182 d	

Fecal and total excretion of ^{14}C were markedly increased in male rats administered cholestyramine (~ 2.7 g/kg/d) in their diet following single intravenous doses of [^{14}C]PFOS. The results suggest that there was significant enterohepatic circulation of PFOS (Johnson and Gibson, 1980, 1984). Cholestyramine administered at 4% by weight in feed to male rats decreased the retention of carbon-14 in liver, plasma, and red blood cells and increased the elimination of carbon-14 via feces after iv dosing with PFOS-^{14}C. Groups of five rats (twelve-week old Charles River CD averaging 320 g) were dosed intravenously with PFOS-^{14}C (mean dose, 3.4 mg/kg). Groups of five control rats were dosed similarly but were not treated with cholestyramine. Rats were sacrificed at 21 days post dose. The mean liver, plasma, and red blood cell concentration as well as fecal and urinary excretion of ^{14}C for cholestyramine-treated

rats were compared to mean control rat values. Mean cholestyramine-treated rat ^{14}C concentrations in liver (9.4µg/g), plasma (0.9µg/ml), and red blood cells (0.3 µg/g) represent a decrease from mean control rat concentrations of 3.8, 7.7, and 6.0 fold, respectively. Fecal elimination (75.9% with cholestyramine treatment) was increased 9.5 fold. The extent of urinary ^{14}C elimination, as a result of the relatively high rate of fecal elimination of ^{14}C was lower in cholestyramine-treated rats. The extent of total elimination of ^{14}C (urine plus feces) was higher in the cholestyramine-treated rats. Since cholestyramine is approved for use in humans as a cholesterol lowering agent, these results in rats support the concept of testing cholestyramine in humans to promote excretion of PFOS (Johnson et al, 1980).

Acute Toxicity

Acute Oral Toxicity: PFOS was administered by gavage in a 20 : 80, acetone: corn oil suspension to five male and five female rats at dose levels of 100, 215, 464, and 1000 mg/kg. Observations of toxicity were made over a 14-day period. All rats in the 1000 and 464 mg/kg dose groups died. The signs before death included hyperactivity, decreased limb tone and ataxia. At 215 mg/kg, three of ten animals died. At 100 mg/kg, no deaths were observed. The combined acute oral LD50 in male and female rats is 251 mg/kg (95% C.I.: 199-318 mg/kg). PFOS is considered moderately toxic on acute oral administration under the conditions of this study (Dean et al., 1978).

Acute Dermal Toxicity: Ten male and ten female albino rabbits were clipped free of hair and an aqueous suspension equivalent to 5 g/kg PFOS was placed over 40% of the total body surface area, occluded with an impervious plastic sheet and left in contact with the skin for 24 hours, then removed. Animals were maintained and observed for a 28-day period after which they were necropsied. Blood samples were obtained on days 1, 7, 14 and 28. Day 0, 7, 14 and 28 body weights were recorded. Hyperactivity was noted in 5 of 10 males on day 6. All animals recovered by day 7 and remained asymptomatic throughout the study period. Weight gains were observed for all rabbits. No visible lesions were noted at necropsy. Analysis of total blood fluoride from day 1 and day 28 blood samples of one male and one female indicated that PFOS was absorbed through the skin, reaching blood concentrations of approximately 130 ppm after 28 days (see section above on absorption). PFOS can be considered practically non-toxic on single dermal contact (O'Malley and Ebbens, 1980).

Acute Inhalation Toxicity: Groups of five male and five female Sprague-Dawley rats were exposed by inhalation for one-hour to nominal exposure concentrations of 24, 7.1, 6.5, 4.9, 2.9, 1.9 and 0.0 milligrams PFOS per liter of air. The rats were observed hourly for the first four hours and daily thereafter for 14 days. All rats

exposed to 24 milligrams per liter died. Partial mortality was produced at nominal exposure concentrations of 7.1 (80%), 6.5 (80%), 4.9 (20%) and 2.9 (10%) milligrams per liter. Symptoms observed during the exposure and post-exposure periods were labored breathing, reduced activity, excessive salivation and lacrimation, mucoid and red nasal discharge, yellow staining of the ano-genital fur, and dried red material on the facial area. The most frequent abnormal necropsy observations were of lung and liver discoloration. Lung discoloration was also observed in a high number of control rats and thus may not be treatment-related. The nominally determined median lethal concentration (LC50) for a one-hour exposure to PFOS was determined to be 5.2 milligrams per liter with 95% confidence limits of 4.4 milligrams per liter and 6.4 milligrams per liter (Rusch and Rinehart, 1979).

Primary Irritation

Ocular Irritation: PFOS was found to be mildly irritating to the eyes of albino rabbits when tested according to standard Federal Hazardous Substances Act guidelines. The ocular irritation was limited to the conjunctivae in the six test rabbits. Irritation was noted at the 1, 24 and 48 hour post-instillation reading times. The maximum irritation score was 9.3 out of a highest possible score of 110 at the 24 hour reading. By 72 hours post-instillation all readings were zero (Biesemeier and Harris, 1974).

Dermal Irritation: PFOS was found to be non-irritating to the skin of albino rabbits when tested under conventional Draize procedures. No signs of dermal irritation were observed in any of the test animals at any time during the study period. The primary skin irritation score was 0.0 out of a highest possible score of 8.0 (Biesemeier and Harris, 1974).

Genotoxicity

Gene Mutation: PFOS was not mutagenic in *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, TA-100 or in *Saccharomyces cerevisiae* strain D4

in a standard plate incorporation assay with or without metabolic activation. (Jagannath and Brusic, 1978).

Chromosomal Effects: PFOS did not cause chromosomal aberrations in an *in vivo* mouse bone marrow micronucleus assay. PFOS in water was administered by oral gavage at 0, 237.5, 475, and 950 mg/kg to five male and five female mice for each dose/ harvest time group. The mice were euthanized 24, 48 and 72 hours after dosing for extraction of bone marrow. No increase in bone marrow polychromatic erythrocytes was observed (Murli, 1996).

Repeated-Dose Toxicity

A number of repeated-dose toxicity studies have been conducted with PFOS and will be discussed in this sub-section. A tabular summary of these studies will be found at the end of this section in Table IV.9 for rodent studies and Table IV.10 for primate studies. In addition to these summary tables, Table IV.12 presents the results of rodent and primate 90-day sub-chronic studies organized by increasing nominal mg/kg dose level.

90-Day Dietary Study in Rats: PFOS was fed in the diet at levels of 0, 30, 100, 300, 1,000 and 3,000 ppm to groups of five male and five female Charles River CD rats for 90 days. These doses represent approximately 0, 2,6, 18, 60 and 180 mg/kg/day. The rats were observed twice daily for overt signs of toxicity and mortality. Individual body weight and sex group food consumption were recorded weekly. Hematological, biochemical and urinalysis studies were conducted prior to commencement of dosing, at one month and at study termination.

At the 30 ppm (approximately 2 mg/kg/day) dosage level, no rats showed any compound related changes in appearance or behavior. Mean body weights were slightly lower when compared to the controls. At one month, one female rat showed a

slightly elevated blood glucose, and one male rat showed a slightly elevated serum alkaline phosphatase. At three months, one male rat had slight to moderate elevations in blood glucose, blood urea nitrogen and gamma-glutamyl transpeptidase activity.

At the 100 ppm (approximately 6 mg/kg/day) dosage level, mean body weight and food consumption was significantly lower than the control group. One male and two females died. Slight increases in creatinine phosphokinase (CPK) and serum alkaline phosphatase activity, slight to moderate increases in blood glucose and blood urea nitrogen, and slight to marked increases in plasma transaminase activities (AST and ALT) were seen at one month. At three months, all rats in the 100 ppm dose group had slight to moderate decreases in hemoglobin, hematocrit and erythrocyte counts, and slight to moderate increases in transaminase activities were seen for two of the three surviving female rats.

At dosage levels of 300, 1,000 and 3,000 ppm (approximately 18, 60 and 180 mg/kg/day) all rats died prior to scheduled termination of the study. Time of death was dose-related. Overt clinical observations of toxicity included emaciation, convulsions, altered posture, red material (right eye and/or mouth), yellow material in the ano-genital region, increased sensitivity to external stimuli and reduced motor activity. Compound-related gross changes such as emaciation and areas of discoloration involving the stomach and liver were observed among treated rats that died prior to sacrifice. Similar changes were also observed in the liver of a few rats sacrificed at termination of study from the 30 and 100 ppm groups.

Morphological changes consisting of centrilobular to midzonal cytoplasmic enlargement (hypertrophy) of hepatocytes and necrosis of liver cells was present in all PFOS dose groups. The incidence and relative severity of the above lesions were more evident among male rats. In addition, rats from the 300, 1,000 and 3,000 ppm dosage levels displayed compound-related changes involving the primary (thymus,

bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin (Goldenthal et al, 1978a).

Two-Year Dietary Study in Rats, Four and Fourteen Week Results: Results are available from four-week and fourteen-week interim sacrifices in Sprague-Dawley rats which are part of an on-going 104-week dietary study (Covance, 1998). In these sub-studies, groups of five male and five female rats (four and 14-week sacrifice groups) and ten male and ten female rats (14-week sacrifice groups) were exposed to either 0, 0.5, 2, 5, or 20 ppm PFOS in the diet for four or 14 weeks. Clinical observations were made twice daily. Body weights and food consumption were measured once a week. Organ weights were measured at term. Samples were taken at both sacrifices for PCNA, liver palmitoyl CoA oxidase activity, serum and liver PFOS concentrations and at the 14-week sacrifice for hematology, clinical chemistry (including urinalysis), and histopatholgy. During weeks 4 and 14, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry tests from ten animals/sex in Groups 1 through 5. Five animals/sex in Groups 1 through 5 were sacrificed during Week 4; livers were collected and weighed. A portion of the liver was prepared for PFOS analysis, a portion was shipped to Pathology Associates International for hepatocellular proliferation rate (PCNA) measurements, and a portion was used for determination of palmitoyl-CoA oxidase activity. At week 14, necropsies were performed on five animals/sex in Groups 1 through 5. At necropsy, macroscopic observations were recorded, organ weights were obtained, and tissues were placed in fixative as specified by the protocol. In addition, liver samples were collected for PFOS analysis, hepatocellular proliferation rate measurement, and palmitoyl-CoA oxidase determination. Microscopic examinations were done on selected tissues from animals necropsied during week 14. The tissues were adrenals, brain, eyes, kidney, liver, mesenteric lymph node, pancreas, spleen, testes, and ovaries. In addition, microscopic examinations were done on tissues from animals that died or were sacrificed due to poor health.

There were relatively few statistically significant or otherwise notable differences for clinical pathology results between the control and treated groups. Although none of the statistically significant differences for high dose animals were consistent at both testing intervals, it was considered likely that administration of the test material was associated with mildly higher urea nitrogen at Week 14 for males and females fed 20.0 ppm and moderately lower cholesterol and mildly higher alanine aminotransferase at Week 14 for males fed 20.0 ppm. There were no correlative microscopic renal findings for the minor change in urea nitrogen. The findings for alanine aminotransferase and cholesterol were likely associated with the histopathological findings of hepatocellular hypertrophy and vacuolation.

Of uncertain relationship to the test material was mildly, but statistically, higher absolute neutrophil count for males fed 20.0 ppm. Females were unaffected, and there were no correlative microscopic findings for this small difference.

All other statistically significant differences for clinical pathology results between the control and treated groups were considered incidental. Lower glucose at Week 4 for high-dose males and lower aspartate aminotransferase at Week 4 for high dose females were considered incidental because they were not present at Week 14. Higher albumin at Week 14 for high dose females was considered incidental because the low dose females had a similar, but higher, statistically significant difference for albumin.

Table IV.2 and IV.3 summarize key clinical pathology results at 14 weeks for males and females, respectively.

Table IV.2 Clinical chemistry and hematology in Male rats at week 14 (n=10)

	Average Value and Std Dev. by Dose Group (ppm PFOS in Diet)					ANOVA
Parameter	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	Pr > F
ALB	5 ± 0.17	5 ± 0.2	4.8 ± 0.35	5 ± 0.3	5 ± 0.16	0.32
ALT (SGPT)	38 ± 31	42 ± 7	40 ± 6	51 ± 15	81 ± 74	0.28
AST (SGOT)	109 ± 31	121 ± 14	139 ± 14	137 ± 10	159 ± 77	0.34
CHOL	64 ± 13	53 ± 22	50 ± 16	57 ± 8	36 ± 18*	0.13
GLU	103 ± 8	104 ± 13	83 ± 15	93 ± 8	92 ± 14	0.062
N_SEG	1 ± 0.4	1.2 ± 0.3	0.94 ± 0.2	1.3 ± 0.46	1.7 ± 0.3	NA
UN	13 ± 2	14 ± 2	14 ± 2	14 ± 0	17 ± 1*	0.0007
PCoAO	4.6 ± 1.34	4.8 ± 3.3	5.4 ± 3	1.8 ± 1.8	5.4 ± 1.9	0.14

* Mean significantly different (Dunnetts' T-test, $p < 0.05$) than the control group value

Table IV.3 Clinical chemistry and hematology in female rats at week 14 (n=10)

	Average Value and Std Dev. by Dose Group (ppm PFOS in Diet)					ANOVA
Parameter	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	Pr > F
ALB	5.5 ± 0.25	6.1 ± 0.5*	5.7 ± 0.17	6.2 ± 0.2*	6.1 ± 0.24*	0.0057
ALT (SGPT)	34 ± 2	39 ± 11	30 ± 5	36 ± 5	33 ± 5	0.24
AST (SGOT)	103 ± 21	108 ± 23	98 ± 8	109 ± 22	79 ± 9	0.092
CHOL	78 ± 19	79 ± 14	70 ± 12	68 ± 19	65 ± 16	0.64
GLU	102 ± 16	100 ± 12	105 ± 3	90 ± 8	100 ± 7	0.31
N_SEG	0.8 ± 0.36	0.9 ± 0.4	0.53 ± 0.13	0.56 ± 0.1	0.64 ± 0.1	NA
UN	14 ± 1	16 ± 4	14 ± 2	16 ± 1.7	16 ± 1.8	0.26
PCOAO	1.8 ± 1.6	3.0 ± 2.6	1 ± 0.8	1.6 ± 2.6	5 ± 2.9	0.10

* Mean significantly different (Dunnetts' T-test, $p < 0.05$) than the control group value

Body and Organ Weights: Terminal body weights at 14 weeks for the 2 ppm dose group were reduced in comparison to controls by 4.5% in the males and 11.7% in the females. Absolute and relative liver weights were significantly increased by approximately 35% in the males given 20.0 ppm PFOS in the diet. In females given

20.0 ppm, the absolute liver weights were increased by approximately 30% over control values, but only the liver-to-body weight percentage was significantly increased. The absolute spleen weight was significantly decreased in the females given 20.0 ppm, as was the absolute lung weight in females given 2.0, 5.0, or 20.0 ppm. Spurious significant increases in left thyroid/parathyroid-to-body weight ratios were seen in females given 5.0 or 20.0 ppm.

Macroscopic Observations: There were no macroscopic observations that could be attributed to the administration of the test material.

Microscopic Observations: Test material related histomorphologic changes were limited to the liver in the males given 5.0 or 20.0 ppm and in the females given 20.0 ppm. The changes consisted of hypertrophy of hepatocytes in centrilobular areas, and midzonal to centrilobular hepatocytic vacuolation. The incidence and severity of the changes tended to be greater in the males.

There were no apparent test material-related lesions in the remaining tissues examined.

Serum and liver concentrations of PFOS increased with dose and with length of exposure, and liver values were significantly higher than corresponding serum values. These analytical results are presented in Tables IV.4 and IV.5 for serum and liver, respectively.

Table IV.4 Mean Serum PFOS Concentrations (ppm) After 4 and 14 Weeks Dietary Exposure

Serum [PFOS] in ppm by Dietary Dose Group (ppm PFOS in Diet) n = 5 per sex per dose group						
Time Interval	Dose Group	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
4 Weeks	Male	< MDL**	1.14 ± 0.07	5.05 ± 1.2	8.95 ± 2.31	46.4 ± 8.46
	Female	0.04 ± 0.003	1.90 ± 0.22	7.50 ± 0.053	14.3 ± 1.85	59.4 ± 8.46
14 Weeks	Male	< MDL**	4.22 ± 0.83	17.9 ± 1.23	45.6 ± 5.73	152 ± 13.3
	Female	2.45 ± 4.19*	6.65 ± 1.08	26.9 ± 2.26	62.9 ± 6.19	216 ± 21.9

*0 ppm female [PFOS] in serum at 14 weeks without one outlying value (n=4) is 0.59 ± 0.59 ppm.
**MDL = Method Detection Limit, which was 15 ppb.

Table IV.5 Mean Liver PFOS Concentrations (ppm) After 4 and 14 Weeks Dietary Exposure

Liver [PFOS] in ppm by Dietary Dose Group (ppm PFOS in Diet) n = 5 per sex per dose group						
Time Interval	Dose Group	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
4 Weeks	Male	0.11 ± 0.74	11 ± 1.70	33 ± 8.0	49 ± 12	295 ± 45
	Female	0.11 ± 0.05	8.6 ± 0.57	25 ± 6.2	80 ± 17	357 ± 43
14 Weeks	Male	0.44 ± 0.05	26 ± 4.4	77 ± 7.5	387 ± 33	600 ± 116
	Female	1.58 ± 2.1	19 ± 3.4	68 ± 3.0	362 ± 26	618 ± 50

90-Day Drinking Water Study with Lithium Perfluorooctane Sulfonate (LPOS) in Rats: The lithium salt of perfluorooctane sulfonate was administered to male and female Sprague-Dawley rats in drinking water for 90 consecutive days. With the exception of controls, male and female doses did not directly correspond and each dose group was either ten males or ten females. Female LPOS doses, expressed as mg/kg body weight, were: 0 (two separate controls, one added later as a concurrent control for the 0.6 mg/kg dose group); 0.02; 0.06; 0.2; and 0.6 mg/kg (added later). Male LPOS doses, also expressed as mg/kg body weight, were: 0; 0.3; 1.0; and 3.0.

Clinical observations included weekly body weight, daily observation, eye examination (day 0 and one week prior to termination), and food and water consumption. Clinical chemistry was performed on blood samples taken at termination. Gross pathology and organ weights were noted at sacrifice and histopathology was performed on preserved tissues.

All rats gained weight in the course of the study. There were no compound-related early deaths.

Clinical pathology demonstrated effects on hematologic and serum chemistry parameters in the mid-dose and high-dose males as well as the high-dose females. Significant hematologic effects in male rats were: 1) decreased hemoglobin, hematocrit and RBC (0.3 and 1.0 mg/kg dose groups); 2) decreased mean corpuscular hemoglobin content (3.0 mg/kg dose group); and 3) higher eosinophil counts (0.3 mg/kg dose group). Significant hematologic effects in female rats were: 1) decreased hematocrit and RBC count (0.6 mg/kg dose group); decreased mean corpuscular volume (0.02 and 0.2 mg/kg dose groups); 3) decreased mean corpuscular hemoglobin and mean corpuscular hemoglobin content (0.02, 0.06 and 0.2 mg/kg dose groups); and 4) increased mean corpuscular hemoglobin and mean corpuscular hemoglobin content (0.6 mg/kg dose group). These hematologic effects did not have a consistent dose-related pattern. Significant serum chemistry effects in male rats were: 1) an elevation in alkaline phosphatase and blood urea nitrogen and decreased cholesterol (1.0 and 3.0 mg/kg dose groups); 2) increased total bilirubin (3.0 mg/kg dose group) and 3) decreased triglycerides (0.3, 1.0 and 3.0 mg/kg dose groups). There were no significant treatment-related serum chemistry effects in female rats.

Gross pathology revealed the following organ-weight effects in male rats: 1) increases in absolute liver weights and liver weight relative to brain weight and liver weight relative to body weight (1.0 and 3.0 mg/kg dose group) and 2) decreased absolute heart weight, decreased heart weight relative to body weight and increased brain

weight, kidney weight and testes weight relative to body weight (3.0 mg/kg dose group). Gross pathology revealed the following organ-weight effects in female rats: 1) increases in absolute liver weights and liver weight relative to body weight (0.06, 0.2 and 0.6 mg/kg dose groups); 2) increased liver weight relative to brain weight (0.2 and 0.6 mg/kg dose groups); 3) decreases in absolute heart weight, heart weight relative to brain weight and heart weight relative to body weight (0.02, 0.06, 0.2 and 0.6 mg/kg dose groups); 4) increased brain weight relative to body weight (0.02 and 0.06 mg/kg dose groups) and 5) increased kidney weight relative to body weight (0.2 mg/kg dose group). With the exception of liver, these organ weight changes were related to reduced body weight gain and were not associated with histopathologic alterations. The dose-related increases in liver weights and reductions in serum cholesterol and triglycerides are supported by the histopathologic finding of hepatic vacuolization in the 1.0 and 3.0 mg/kg male rats.

90-Day Oral Gavage Study in Rhesus Monkeys: In an initial study, five groups consisting of two male and two female rhesus monkeys initially weighing between 2.75 and 4.10 kg were given daily doses of 0, 10, 30, 100 and 300 mg PFOS / kg by gastric intubation (gavage) as distilled water suspension. This study was terminated after 20 days due to the death of all PFOS-dosed monkeys (Goldenthal, 1978). Time of death was dose-related. Monkeys in the 300 mg/kg/day dose group died between the second and fourth day. The 100 mg/kg dose group died between the third and fifth day. At 30 mg/kg, deaths occurred between the seventh and tenth day. The 10 mg/kg dose group died between days 11 and 20. All PFOS-treated monkeys lost weight. Clinical observations of toxicity were similar for all PFOS dose groups, and time of onset of toxic effects was dose-related. These toxic symptoms included anorexia, slight to severe decreased activity, frothy or food-like emesis, and occasional diarrhea. Prior to death, body and limb stiffening, general body tremors, convulsions and prostration were observed. The 10 mg/kg dose group included one monkey who had black stools and one who developed facial erythema. Gross pathology revealed a

yellowish-brown discoloration of the livers of several monkeys in the 100 mg/kg and 300 mg/kg dose groups. Organ weights appeared within normal limits; however, due to early deaths of PFOS-treated monkeys and the reuse of control monkeys on a follow-up study at lower dose levels, concurrent control data was not available. Histopathologic examination of tissues from treated monkeys did not reveal any consistent observations which could be directly related to an effect of PFOS. Congestion and lipid depletion of the adrenal cortex was seen in all dose groups and was considered agonal. The study was terminated after the death of the last 10 mg/kg dose-group monkey.

After termination of the initial study after early deaths of all PFOS-treated monkeys, the control group monkeys were used in a follow-up study at lower doses (Goldenthal etal, 1978b). In this study, five groups consisting of two male and two female rhesus monkeys initially weighing between 2.55 and 3.75 kg were given daily doses of 0, 0.5, 1.5, and 4.5 mg PFOS / kg by gastric intubation (gavage) as distilled water suspension for 90 days. The monkeys were observed twice daily for general physical appearance, behavior and other clinical signs of toxicity. Body weights were recorded weekly. Hematological and biochemical studies and urinalysis were conducted once in the control period and at the end of the first and third month of the study.

The monkeys treated at the 4.5 mg/kg/day dosage level died or were sacrificed in extremis between weeks 5 and 7 of the study. These monkeys exhibited gastrointestinal symptoms including anorexia, emesis, black stool and dehydration from the first or second day of study. These monkeys also exhibited decreased activity and showed marked to severe rigidity, convulsions, generalized body trembling, prostration and loss of body weight prior to death. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.70 kg at week 5 of the study. All monkeys at the 4.5 mg/kg/day dosage level had decreased serum cholesterol values and serum alkaline phosphatase activity at one month.

All monkeys at the 1.5 mg/kg/day dosage level survived to the end of the study. These monkeys exhibited slightly decreased activity from the first week of the study which occasionally became moderate to marked. In addition, they occasionally had black stools, diarrhea, mucous in the stool and bloody stool and exhibited dehydration or general body trembling at the end of study. The monkeys from this group had a slight decrease in mean body weight. Slight decreases in serum alkaline phosphatase activity and serum inorganic phosphate concentrations were evident at the end of the study in addition to a marked decrease in serum cholesterol.

All monkeys at the 0.5 mg/kg/day dosage level survived to the end of the study. Monkeys at this dosage level exhibited an occasional soft stool, diarrhea, anorexia and emesis, all of which also occurred occasionally in the control group. Slightly decreased activity was noted intermittently in three monkeys at this dosage level. At three months of study a statistically significant decrease in the serum alkaline phosphatase activity was noted in the males as compared to control values; however, the toxicologic significance of this finding is questionable when compared to individual pre-dose values. The 0.5 mg/kg dose has been considered to present a LOEL by past reviewers. Recent evaluation of the study by 3M toxicologists lend to a conclusion that this dose more likely represents a NOAEL, if not a NOEL.

No treatment-induced gross or microscopic pathological lesions were seen in tissues other than the adrenals, pancreas, and submandibular salivary glands of male and female rhesus monkeys at the 4.5 mg/kg/day dosage level. Microscopically, the adrenals from male and female monkeys at the 4.5 mg/kg/day dosage level had compound-related marked diffuse lipid depletion; the pancreas from male and female monkeys at the 4.5 mg/kg/day dosage level had compound-related moderate diffuse atrophy of exocrine cells; the submandibular salivary glands from male and female monkeys had compound-related moderate diffuse atrophy of the serous alveolar cells. No statistically significant variations in sex group mean weights of organs occurred between the control and experimental groups.

Table IV.6 summarizes the individual cholesterol values for all dose groups.

Table IV.6: Longitudinal analysis of serum cholesterol and PFOS concentrations of individual male and female rhesus monkeys during 90 days dosing with either 1.5 or 4.5 mg/kg PFOS (n = 2 per sex per dose group) (Goldenthal etal, 1978b).

Individual Serum Cholesterol (mg /100 ml) by Study Period and Cumulative Dose (CD in mg/kg)

Dose Group	Individual	Pre-Dosing	1 Month	3 Months	% of Pre-Dosing Value at Term
		CD = 0	*CD = 0*	*CD = 0*	
0	♂ (ID # 7355)	192	212	179	93.2
mg/kg/day	♂ (ID # 7358)	174	184	144	82.8
Dose Group	♀ (ID # 7368)	155	188	144	92.9
Monkeys	♀ (ID # 7372)	204	208	185	90.7
		CD = 0	*CD = 15*	*CD = 45*	
0.5	♂ (ID # 7463)	182	208	160	87.9
mg/kg/day	♂ (ID # 7483)	161	192	143	88.8
Dose Group	♀ (ID # 7466)	208	230	178	85.6
Monkeys	♀ (ID # 7504)	208	211	160	76.9
		CD = 0	*CD = 45*	*CD = 135*	
1.5	♂ (ID # 7462)	196	222	132	67.3
mg/kg/day	♂ (ID # 7486)	174	204	112	64.4
Dose Group	♀ (ID # 7500)	172	154	128	74.4
Monkeys	♀ (ID # 7501)	236	184	96	40.7
		CD = 0	*CD = 135*	**No Survivors**	
4.5	♂ (ID # 7484)	186	76	-	40.9
mg/kg/day	♂ (ID # 7485)	194	97	-	50.0
Dose Group	♀ (ID # 7502)	182	128	-	70.3
Monkeys	♀ (ID # 7503)	170	96	-	56.4

28-Day Range-Finding, Oral Capsule-Dosing Study in Cynomolgus Monkeys: Male and female cynomolgus monkeys weighing 2.1 to 2.4 kg were given capsules placed directly in the stomach that contained PFOS at either 0.0 mg/kg/day (two males and two females), 0.02 mg PFOS/kg/day (three males and three females), or 2.0 mg/kg/day (one male and one female) for 28 days in a range-finding study to determine doses for a six-month chronic oral capsule-dosing study (Thomford, PJ, 1998). Blood was collected for clinical chemistry on study days - 7 (baseline values) , 2, 7, 14 and 29. In addition to standard hematologic parmeters and serum chemistry determinations, sex and thyroid hormones, cholecystokinin (CCK) and pancreatic amylase were measured. At the same time points and on day 3, blood was also obtained for determination of serum PFOS concentration. Tissues were obtained at necropsy, weighed, fixed and prepared for histopathologic analysis. In addition to histopathologic samples, liver specimens were obtained for analysis for proliferating cell nuclear antigen (PCNA), determination of PFOS concentration, and determination of palmitoyl CoA oxidase activity. Serum PFOS concentrations increased with a high degree of linearity at both dose levels, with no difference between males and females and at a linear rate of 5.3 ppm serum PFOS per mg/kg (for details please see subsection on toxicokinetics). At the end of the 28-day dosing period, serum PFOS concentration in the 0.02 mg/kg/day dose group reached approximately 3 ppm and in the 2.0 mg/kg/day dose group, serum concentrations reached approximately 300 ppm. The only treatment-related effect observed in the study was a dramatic reduction in serum cholesterol in the male and female that received a dose of 2.0 mg/kg/day. Serum cholesterol dropped from baseline values of 150 and 141 mg/dl for the male and female, respectively, to 91 and 62 mg/dl at termination on day 29. The first evidence of a significant decrease occurred between day 2 and day 7 for the female, with a day 2 value of 136 mg/dl and a day 7 value of 117 mg/dl. The male cholesterol value fell from to 151mg/dl on day 2 to 137 on day 7. The day 7 values for the male and female corresponded to a serum PFOS concentration of 72 ppm. This cholesterol data and corresponding cumulative dose and serum PFOS concentrations are summarized in Table IV.7. There were no other significant findings.

Table IV.7: Longitudinal analysis of serum cholesterol and PFOS concentrations in male and female cynomolgus monkeys (n = 1 per sex) during 28 days dosing with 2.0 mg/kg PFOS.

Study Day	-7	2	7	14	29
Cumulative Dose (mg/kg)	0	2	12	26	56
♂ Serum Cholesterol (mg/dl)	150	151	137	132	91
♂ [PFOS] in serum (ppm)	0.013	12.6	71.8	129	313
♀ Serum Cholesterol (mg/dl)	141	136	117	107	62
♀ [PFOS] in serum (ppm)	0.014	14.4	72.7	143	299

Six-Month Oral (Capsule) Study in Cynomolgus Monkeys

Unaudited clinical pathology and clinical observations through 90 days of dosing are available from an ongoing six-month oral (capsule) dosing study in cynomolgus monkeys (Covance Study Number 6329-223, in progress). In this study cynomolgus monkeys are being dosed by capsule with either 0, 0.03, 0.15, or 0.75 mg/kg/day PFOS for a period of six months. Dose groups include six monkeys per sex per group with the exception of the 0.03 mg/kg/day dose group which includes four monkeys per sex. The only significant finding through 90 days is a reduction in serum total cholesterol in the males and females of the high dose group. While serum PFOS concentrations have not yet been analyzed and reported, previous experience in the range-finder for this study suggests that the serum concentrations are expected to center on approximately 350 ppm PFOS as 90 days for the high-dose monkeys.

Reproductive and Developmental Toxicity

This sub-section discusses the available information on developmental and reproductive toxicity. A summary of this data in tabular form can be found at the end of this section in Table IV.11.

Oral Developmental Toxicity (Teratology) in Rats: A rat PFOS oral teratology study was conducted at Riker Laboratories (Gortner etal, 1980). Dose levels (oral)

given to the pregnant rat dams were 0, 1, 5, and 10 mg/kg. Maternal toxicity (reduced weight gain) occurred at the high dose of 10 mg/kg on days 6 through 15 of gestation. Evidence of fetal toxicity was not found at any dose level. No skeletal and soft tissue teratogenic changes were found at any dose level with one exception. A change in the lens of the eye was found in all dose groups including the control but the incidence in high dose group was significantly higher. This change was reported out as a developmental eye abnormality and the summary of the report states the compound was teratogenic. An outside consultant and teratology expert, Dr. E. Marshall Johnson from Jefferson Medical College, visited 3M and reviewed the rat pup eye specimens in question. He concluded that the eye/lens changes were, in fact, sectioning artifacts and not compound related teratology abnormalities. Thus, the weight of the evidence indicates that PFOS does not cause teratogenic effects in rats when dosed at levels which are not maternally toxic. The lens change observed in rat pups in Riker Laboratories studies was a sectioning artifact and was not found upon repeat studies at independent laboratories.

Oral Developmental Toxicity (Teratology) in Rats: In a subsequent study, PFOS (suspended in corn oil) was administered on gestational days 6-15 by oral gavage to groups of 25 pregnant Sprague-Dawley CD rats at doses of 0 (control), 1, 5, and 10 mg/kg/day (Wetzel, etal, undated). Severe maternal toxicity occurred in the 5 mg/kg and 10 mg/kg dose groups, as evidenced by significant reductions in mean body weight gain, terminal body weight minus gravid uterine weight and food consumption compared to control dams, actual losses in body weight on commencement of treatment among numerous dams and death in two dams in the 10 mg/kg dose group prior to gestational day 20. Mean body weight gains (days 0-20) at 5 and 10 mg/kg were 104 ± 35 (S.D.) and 34 ± 73 (S.D.), respectively, as compared to 125 ± 24 (S.D.) in the control group. Mean food consumption values (days 0-20) at 5 and 10 mg/kg were 363 ± 60 (S.D.) and 264 ± 90 (S.D.), respectively, as compared to 421 ± 28 for the control group. Mean terminal body weight minus gravid uterine weight at 5 and 10 mg/kg was 293 ± 28 (S.D.) and 241 ± 60 (S.D.), respectively, as compared to

321 ± 23 (S.D.) in the control group. Clinical signs in surviving dams included hunching, lower body weight, alopecia, rough haircoat, anorexia. Gastrointestinal and kidney lesions were noted in the high-dose dams.

Treatment-related fetal effects that were attributed to maternal toxicity included: increased resorptions and fetal death, decreased fetal body weight, delayed skeletal ossification, cleft palate, subcutaneous edema and cryptorchism (undescended testicles). These effects occurred primarily in the high-dose group. The maternal and fetal NOAELs for this study were both 1 mg/kg/day.

Oral Developmental Toxicity (Teratology) in Rabbits: A final draft report of an oral developmental toxicity study in rabbits was recently received (York, 1998). In this study, dose groups of 22 pregnant new zealand white rabbits were dosed on days 7 through 20 of gestation with either 0, 0.1, 1.0, 2.5, or 3.75 mg/kg/day PFOS.

Maternal toxic effects included: 1) decreased body weight at the highest three dose levels with a minimal effect at the 1.0 mg/kg dose; 2) decreased food consumption at the highest two doses; 3) frequent scant feces at the highest dose, and 4) increased abortions at the highest two doses.

Fetal toxic effects included reduced fetal weight and an increase in delayed ossification at the highest two doses. No teratogenic events were observed in the study.

Based on this draft final report, PFOS was not teratogenic under conditions of the study and the maternal and fetal NOELs are 0.1 mg/kg/day and 1.0 mg/kg/day, respectively.

Two-Generation Reproductive Toxicity in Rats by Oral Gavage: Interim results are available from an on-going two-generation reproduction study in rats by oral gavage (Argus, 1998). In this study, groups of 35 male and 35 female rats were

exposed to 0, 0.1, 0.4, 1.6 and 3.2 mg/kg by daily oral intubation six weeks prior to and during mating. For the females, treatment continued during gestation, parturition and lactation.

A pre-mating reduction in mean body-weight gain as compared to controls occurred in females and possibly in the males at the high dose level. At the 3.2 mg/kg dose level, male body-weight gain was 97.2 % of control and female body-weight gain was 91.5 % of control. There was no effect on the number of pregnancies.

During gestation, females showed a reduction in mean body-weight gain at the three highest dose levels, reaching 86.9% of the control at 3.2 mg/kg. A group of ten dams per dose group was sacrificed on day 10 of gestation. No increases in resorptions occured, and there were no decreases in the number of implantations or number of live fetuses.

At parturition in the 3.2 mg/kg high-dose group, the mean number of pups delivered was decreased compared to the control (10 versus 14) and the percent of pups delivered stillborn was increased significantly (24.9 % as compared to 2.2 %.) Survival of pups during days one through four of lactation was severely affected at 1.6 and 3.2 mg/kg (66 % and 0% survival, respectively.) Most deaths at the high dose occured within the first 24 hours after birth. Surviving pups in the 1.6 mg/kg dose group showed severely depressed mean body-weight gains through day 21 of lactation (72.1 % of controls.)

A series of follow-up studies are to be initiated in November, 1998 to gain a better understanding of the reduced perinatal survival at the high dose levels. These will include a complete cross-fostering study, and two pharmacokinetic studies with pregnant dams.

The post-weaning F_1 generation currently exhibits no signs of toxicity at 0.1 and 0.4 mg/kg/day.

Mechanistic Studies

The specific mechanisms relating to PFOS toxicity are not completely understood. Several studies provide clues to the potential operative mechanism(s) of toxicity. Effects on lipid and lipoprotein processing, cholesterol synthesis and bioenergetics have been studied.

Mitochondrial Bioenergetics: Studies in isolated rat liver mitochondria at PFOS concentration in the range of 10 μM demonstrate: 1) detergent type effects on mitochondrial membranes; 2) stimulation of mitochondrial respiration, and 3) fluidization of the inner mitochondrial membrane (Wallace and Starkov, 1998).

Interference with Fatty Acid Binding and Transport: Interference of PFOS (1-10 μM) with endogenous fatty acid binding to carrier protein substrates, liver fatty acid binding protein (L-FABP) and albumin (BSA) has been investigated and show a 66% reduction of initial fluorescence when added to solutions containing 1mM L-FABP and 1mM DAUDA and an IC_{50} of 4.9 μM (Nabbefeld et al., 1998; Nabbefeld, 1998).

Peroxisome Proliferation: Liver biochemical effects associated with peroxisome proliferation have been investigated in two published studies.

Sohlenius et al. (1993) exposed mice to 30 mg/kg/day PFOS for five days (0.05% in diet). In addition to weight loss, increases in each of the following hepatic parameters were observed: 1) relative liver weight (slight elevation); 2) mitochondrial and microsomal protein; 3) palmitoyl-CoA oxidation; 3) catalase in mitochondrial and cytosolic fractions; 4) glutathione transferase; 5) epoxide hydrolase and 6) DT-diaphorase, Ω- and Ω-1-hydroxylation (Sohlenius et al., 1993).

Ikeda et al. (1987) exposed male rats were to 0.02% PFOS in the diet for 2 weeks. The prominent induction of peroxisome proliferation was demonstrated by electron microscopy. Activities of catalase, fatty acyl-CoA oxidizing system, carnitine acetyl transferase and cytochrome P450s responsible for the Ω-hydroxylation of lauric acid were increased (Ikeda et al., 1987).

Hypolipidemia: The mechanism of the hypolipidemic effect of PFOS has been studied. Rats were fed 12 mg/kg/day for 7 – 14 days (0.02% in diet). Decreased body weight, increased liver weight, increased liver triacylglycerol, increased liver free cholesterol, decreased liver cholesterol ester, decreased serum cholesterol and triacylglycerols were observed. Hepatocytes isolated from treated rats showed reduced synthesis of cholesterol from acetate, pyruvate and hydroxymethylglutarate but not from mevalonate, increased oxidation of palmitate and reduced fatty acid synthesis. Activities of liver hydroxymethyl glutaric acid-CoA reductase and acyl-CoA:cholesterol acyltransferase were reduced. These results suggest that the hypolipemic effect of PFOS may be due to impaired production of lipoprotein particles due to reduced synthesis and esterification of cholesterol together with enhanced oxidation of fatty acids in the liver (Haughom and Øystein, 1992).

On-Going Research Program

A number of studies were initiated in 1998 to gain better insight into the potential health hazards of PFOS and to provide a strong foundation for risk characterization. These studies are discussed in Section VI, and outlines of them can be found in the Appendix.

Table IV.8: Summary of Rodent Repeated-Dose Studies

Study	Species (strain)	Dose (units)	*n* (Male = M Female = F)	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology
90-Day Dietary (Goldenthal et al., 1978a)	Rats/CD	0 (ppm)	5 M / 5 F	No effect
		30	5 M / 5 F	↓ body wgt, ↑ glutamate-pyruvate transaminase & glutamate-oxalacetate transaminase, liver discoloration
		100	5 M / 5 F	3 deaths; ↑sensitivity to stimuli, red material around the eyes or mouth, ↓ food consump., ↑ CPK, alk phos, glucose & BUN, ↓ hemoglobin, hematocrit, erythrocyte count, reticulocyte count (in females) and leucocyte count, liver enlargement, necrosis and hepatocellular hypertrophy and stomach discoloration and hemorrhage
		300	5 M / 5 F	Death; emaciation, convulsions, stomach mucosal hyperkeratosis, bone marrow hypocellularity, thymic follicular atrophy, splenic lymphoid follicular atrophy, atrophy of mesenteric lymph nodes, atrophy of villi in small intestines, skeletal muscle atrophy and dermal acanthosis and hyperkeratosis.
		1000	5 M / 5 F	Death; hunched posture
		3000	5 M / 5 F	Death; hypoactivity
14-Week Dietary (Part of on-going 2-yr study)	Rats (Sprague Dawley	0 (ppm)	10 M / 10 F	No effect
		0.5	10 M / 10 F	
		2	10 M / 10 F	
		5	10 M / 10 F	
		20	10 M / 10 F	↓ body wgt (♂ & ♀), ↓ cholesterol (♂),↑ liver wgt (♂ & ♀), enlarged & vacuolated liver cells, ↑ Palmitoyl CoA oxidase activity
90-Day Drinking Water (Limoges, 1995)	Rats (Sprague Dawley)	0 (mg/kg/d)	10 M / 10 F	No effect
		0	10 F	No effect
		0.02	10 F	No effect
		0.06	10 F	↑ liver weight
		0.2	10 F	↑ liver weight
		0.6	10 F	↑ liver weight, hematology and serum chemistry effects
		0.3	10 M	↓ serum triglycerides
		1.0	10 M	↑ liver weight, ↓ serum cholesterol and triglycerides, hepatic vacuolization
		3.0	10 M	↑ liver weight, ↓ serum cholesterol and triglycerides, hepatic vacuolization

Table IV.9: Summary of Primate Repeated-Dose Studies

Study	Species (strain)	Dose (units)	*n* (Male = M Female = F)	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology
90-Day Gavage (Goldenthal et al., 1978)	Rhesus Monkey	0 (mg/kg/d)	2 M / 2 F	No effect
		10	2 M / 2 F	Death within 11 - 20 days, weight loss, weakness anorexia, ↓ activity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
		30	2 M / 2 F	Death within 7 - 10 days; weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
		100	2 M / 2 F	All dead in 3 – 5 days, weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
		300	2 M / 2 F	All dead in 2 – 4 days, weight loss, marked weakness, anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
90-Day Gavage (Goldenthal et al., 1978b)	Rhesus Monkey	0 (mg/kg/d)	2 M / 2 F	No effect
		0.5	2 M / 2 F	Slight & intermittent ↓ activity in 3 of 4 monkeys
		1.5	2 M / 2 F	Blood & mucous in stools, diarrhea, dehydr., tremors, ↓ body wgt, ↓ cholesterol, marked ↓ activity
		4.5	2 M / 2 F	marked ↓ cholesterol Death within 7 weeks; black or bloody stool, dehydration, rigidity, convulsions, prostration, decreased serum cholesterol, diffuse lipid depletion of adrenals, atrophy of pancreatic exocrine cells and atrophy of submandibular salivary gland serous alveolar cells, decreased serum alkaline phosphatase, increased SGOT.
28-Day Capsule Range-finding	Cyno. Monkey	0 (mg/kg/d)	2 M / 2 F	No effect
		0.02	3 M / 3 F	No Effect
		2.0	1 M / 1 F	↓ cholesterol (♂ & ♀)

Table IV.10: Summary of Developmental and Reproductive Studies

Study	**Species (strain)**	**Dose (units)**	***n* (Male = M Female = F)**	**Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology**
Teratology (Riker, 1980)	Rat (CD)	0 (mg/kg/d) 1 5 10	20 F (preg.) 20 F (preg.) 20 F (preg.) 20 F (preg.)	No effect No effect No effect ↓ Body wgt gain
Teratology (Wetzel, 1980 & Wetzel, et al., 1983)	Rat (CD)	0 (mg/kg/d) 1 5 10	25 F (preg.) 25 F (preg.) 25 F (preg.) 25 F (preg.)	No effect No effect ↓ maternal (♀), body wgt gain, ↓ food consumption, thinness, hunching, rough hair coat, anorexia, ↓ pup weight ↑ subcutaneous edema, cleft palate & cryptorchism, ↓ maternal (♀), body wgt gain, ↓ food consumption, slight ↓ pups per litter, ↓ pup weight
Two-Gen Reproduct. (in progress, 10/98)	Rat (CD)	0 (mg/kg/d) 0.1 0.4 1.6 3.2	35 M / 35 F 35 M / 35 F 35 M / 35 F 35 M / 35 F 35 M / 35 F	No effect No effect No effect ↑ stillborn pups & ↓ pup survival, (♀), body weight gain ↓ body wgt gain (♀), slight ↓ body wgt gain (♂), ↑ stillborn pups & ↓ pup survival

Table IV.11: 90-Day Sub-Chronic Study Results Organized by Increasing Nominal mg/kg Dose Level

Dose as mg/kg/day	Dosage form	Species	Outcome
0.02	Drink. Water	F rat	No effect (Limoges, 1995)
0.06	Drink. Water	F rat	No adverse effect; increased liver weight (Limoges, 1995)
0.20	Drink. Water	F rat	No effect; increased liver weight (Limoges, 1995)
0.30	Drink. Water	M rat	Reduced serum triglycerides (Limoges, 1995)
0.50	Gavage	Monkey	anorexia, emesis, diarrhea and slightly decreased serum alkaline phosphatase. (Goldenthal et al., 1978b)
0.60	Drink. Water	F rat	Hematology and serum chemistry effects; increased liver weights (Limoges, 1995)
1.0	Drink. Water	M rat	Reduced serum cholesterol and triglycerides; hepatic vacuolization; increased liver weights (Limoges, 1995)
1.5	Gavage	Monkey	hypoactivity, tremors, weight loss, decreased serum alkaline phosphatase and inorganic phosphate (Goldenthal et al., 1978b)
2.0	Dietary	Rat	Weight loss, elevated plasma glutamate-pyruvate transaminase. elevated plasma glutamate-oxalacetate transaminase and liver discoloration. (Goldenthal et al., 1978a)
3.0	Drink. Water	M rat	Reduced serum cholesterol and triglycerides; hepatic vacuolization; increased liver weights (Limoges, 1995)
4.5	Gavage	Monkey	Death within 7 weeks; black or bloody stool, dehydration, rigidity, convulsions, prostration, decreased serum cholesterol, diffuse lipid depletion of adrenals, atrophy of pancreatic exocrine cells and atrophy of submandibular salivary gland serous alveolar cells, decreased serum alkaline phosphatase, increased SGOT. (Goldenthal et al., 1978b)
6.0	Dietary	Rat	3 deaths; increased sensitivity to external stimuli, red material around the eyes or mouth, decreased food consumption, elevated plasma creatinine phosphokinase, alkaline phosphatase, blood glucose and blood urea nitrogen, decreased hemoglobin, hematocrit, erythrocyte count, reticulocyte count (in females) and leucocyte count, liver enlargement, necrosis and hepatocellular hypertrophy and stomach discoloration and hemorrhage (Goldenthal et al., 1978a).
10	Gavage	Monkey	Death within 11 - 20 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex. (Goldenthal, 1978)
18	Dietary	Rat	Death; emaciation, convulsions, stomach mucosal hyperkeratosis, bone marrow hypocellularity, thymic follicular atrophy, splenic lymphoid follicular atrophy, atrophy of mesenteric lymph nodes, atrophy of villi in small intestines, skeletal muscle atrophy and dermal acanthosis and hyperkeratosis. (Goldenthal et al., 1978a)
30	Gavage	Monkey	Death within 7 - 10 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex (Goldenthal, 1978)
60	Dietary	Rat	Death; hunched posture. (Goldenthal et al., 1978a)
100	Gavage	Monkey	Death within 3 – 5 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex (Goldenthal, 1978)
180	Dietary	Rat	Death; hypoactivity. (Goldenthal et al., 1978a)
300	Gavage	Monkey	Death within 2 – 4 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex. (Goldenthal, 1978)

V. RISK CHARACTERIZATION

The principal issue of concern is the potential health risk associated with the levels of PFOS identified in serum samples from non-occupationally exposed employees, commercially available serum and blood bank samples. The purpose of this section is to evaluate this risk using available human and animal data. Sources of exposure and their control are discussed in a companion document.

As noted previously (Section III), 3M has conducted medical surveillance programs at manufacturing locations where employees are exposed to PFOS or precursor molecules. These employees have PFOS serum levels that range from less than one ppm up to 12 ppm in the most recent testing. The 1997 mean among participating employees at the Decatur, Alabama plant, the U. S. location where PFOS related materials are principally produced, was 2.0 ppm. The best current estimate of serum levels in non-occupationally exposed individuals comes from the pooled samples collected from regionally diverse blood banks. These values ranged from 14 to 56 ppb, and averaged 30 ppb. It should be noted that while this is our best estimate, it cannot be considered representative of the U.S. population since blood donors are not necessarily representative of the U.S. population. In addition this data, since it is from pooled samples, does not reflect the variability and range one would expect to find among individuals.

Analysis of data collected in 1995 and 1997 reveals that the serum concentrations of PFOS identified in production workers are not associated with alterations in the hematological or clinical chemistry parameters that were evaluated. This includes measurement of the parameters that would reflect the pathology found in high dose animal studies (see below). In addition, no differences from normal were detected in ten different hormone levels. These analyses included employees of the 3M Belgium manufacturing facility, where serum levels are slightly lower than Decatur. The findings apply for PFOS serum levels up to 6 ppm, approximately two orders of

magnitude above the upper end of the range seen in the U.S. regional blood bank samples. The mean serum concentration in participating Decatur plant employees in 1997 was 68 times the mean of these blood bank samples.

In 1995, epidemiologists from the University of Minnesota completed a mortality study of the Decatur employee cohort. No higher than expected mortality rates were found for any cancer or for any other cause of mortality. This study involved 1,957 employees who had worked at least one year at 3M's Decatur, Alabama plant, and their mortality experience was traced from 1961 through the end of 1991. Details of this study can be found in Section III.

Animal toxicology data has been reviewed in Section IV. An older data set from rhesus monkeys exists and new data from a rangefinder study in cynomolgus monkeys is available. Serum PFOS concentrations were not measured in the older rhesus monkey studies, but can be estimated based on data from the cynomolgus monkey studies. It is recognized that uncertainties are introduced by such estimates, and that derived numbers need to be confirmed in ongoing or planned studies.

Data from the cynomolgus monkey rangefinder study itself documents that a decrease in cholesterol, an early biological effect in both rodents and primates, was observed at a PFOS serum level of 72 ppm in one of the two high dose animals. In the rhesus monkey studies, lowered cholesterol was observed after 90 days of oral dosing at 1.5 mg/kg/day. Severe toxicity, resulting in death, occurred at doses equal to and greater than 4.5 mg/kg/day, in seven weeks or less. Serum concentrations of PFOS that might have been achieved in the older rhesus monkey studies have been estimated from the cumulative dose - serum concentration of PFOS relationship seen in the recent cynomolgus study. These estimates suggest that severe toxicity occurred at serum levels of 700 to 800 ppm and death at 1000 to 1200 ppm.

Other available toxicity data also provide valuable information. PFOS is not mutagenic in five different bacterial strains. It fails to cause chromosomal aberrations in the mouse bone marrow micronucleus assay. Developmental studies in rats show that the compound causes adverse effects on fetal structural development only at doses that are clearly toxic to the mothers.

Preliminary results from an ongoing two generation reproductive study in rats, through F1 weaning, show no interference with sexual maturation, mating behavior, fertilization, number of resorptions, or litter size. At the highest two dose groups (1.6 and 3.2 mg/kg/day) there was significant perinatal mortality in the F1 generation. This effect was not seen at 0.4 mg/kg/day. The study cannot be used in risk assessment until it is completed and fully evaluated, but is discussed here because we are aware of these interim results. The doses observed to cause effect are in the range where toxicity is observed in 90 day rat studies.

Several 90-day studies in rats, involving PFOS administration over a wide range of doses in the diet, in drinking water, and by stomach tube, reveal that no significant toxic effects are produced at or below a dose of 0.2 mg/kg/day. Above this dose adverse effects on the liver, body weight, and changes in several indicators of fat metabolism are demonstrable.

Possible explanations for the absence of detectable toxicity in production workers are that the workers' serum and tissue levels of PFOS are significantly below those achieved in the animal studies, as estimates would indicate, or human beings are less sensitive to the effects of PFOS than are laboratory animals. Of these two explanations, the former seems more likely, because both primates and non-primates were shown to be susceptible to PFOS exposure in the animal studies, so there is no obvious reason to expect large interspecies differences in sensitivity. The second possible explanation – that humans are less sensitive – cannot be ruled out based on available data. It is also possible that the human studies were insufficiently powerful,

because of sample size limitations, to detect the toxicity of PFOS. This seems unlikely because the animal studies showing toxicity involved even fewer subjects, and, at a given dose, all or most animals responded in similar ways. Sample size would not seem to be a significant factor for evaluating clinical, hematological, or hormonal effects.

Excess mortality, including that associated with cancer, has not been observed in production workers. The experimental data showing that PFOS is not mutagenic (Ames tests) and does not cause chromosomal aberrations (mouse bone marrow micronucleus assay) is consistent with these findings.

Non-occupationally exposed populations appear to exhibit serum levels that are approximately two orders of magnitude lower than those exposed occupationally, more than three orders of magnitude below levels estimated to show minimum biological effects (cholesterol reduction) in primate studies and three to four orders of magnitude below levels associated with significant toxic effects. None of the health effects evaluated and found to be absent in the worker studies would be expected to occur in the non-occupationally exposed populations. The additional information provided by the two generation reproductive study in rats is not interpretable at this time, but the dose at what currently appears to be a no effect level is comparable to doses that produce no effect for other endpoints. The animal data revealing adverse effects are not yet fully useful for quantifying human risks, but estimates from current and ongoing studies suggest that PFOS serum levels achieved in those earlier animal studies with compound related effects must have been greater than those experienced by workers and therefore much greater than those experienced by non-occupationally exposed populations.

The currently available evidence does not suggest there is health risk associated with the levels of PFOS found in the serum of occupationally or non-occupationally exposed employees, blood bank samples or commercially available serum.

Several avenues of further investigation are needed. The long-term consequences of PFOS exposure require additional study. Although the available worker studies do not suggest a health problem or evidence of increased mortality rates, animal data reflecting chronic exposures are not yet available. Such data are recommended because worker studies, involving relatively small populations, are generally not sufficiently powerful to detect small excess risks. A two year PFOS feeding study in rats was started in April of 1998. The utility of the available animal data is limited because serum levels associated with adverse events can only be estimated at this time. This limitation is being remedied by collecting additional animal data, including sufficient kinetic information to assess the relationship between administered doses and serum and tissue levels, as cited above for cynomolgus monkeys, as well as more definitive data on compound distribution and elimination.

There is data suggesting that PFOS can, at sufficiently high doses, induce peroxisome proliferation in rodents and alter metabolic processes in laboratory models. Further study of these effects is desirable to determine whether they can occur at serum and tissue levels relevant to those observed or estimated in humans. Section VI of this report describes additional 3M studies that will address these issues. Further follow-up on worker mortality experience is also underway, including an attempt to better classify workers with regard to PFOS exposure.

Finally, studies are underway to improve knowledge about possible sources of PFOS, and the pathways by which non-occupationally exposed individuals are exposed. Although there is no current evidence that the reported serum levels, and corresponding body burdens, represent a health risk, it is appropriate to identify and act on possible ways to reduce exposure. To date this has been done through reduction of residual monomer levels in products and reduced manufacturing emissions.

VI. CURRENT AND PLANNED RESEARCH

Toxicology Studies

Fluorochemical Study Purposes and Outlines

A number of studies were initiated in 1998 to gain better insight into the potential health hazards of PFOS and to provide a strong foundation for risk characterization. These studies include those conducted with PFOS as well as studies which are conducted with N-Ethyl Perfluorooctane Sulfonamido Ethanol (N-EtFOSE), N-Methyl Perfluorooctane Sulfonamido Ethanol (N-MeFOSE) and other related compounds which are presumed to degrade metabolically to PFOS. N-EtFOSE and N-MeFOSE represent major starting points for additional synthesis and are regulated by FDA as contaminants of indirect food additives. These compounds are also known to be absorbed well from the gastrointestinal tract and will produce PFOS as a major metabolite. Therefore, it is appropriate to use these two compounds as models in further investigating the toxicity of this class of chemicals which have the perfluorooctane sulfonyl moiety as a base. The goals of this research program are as follows:

- To understand the relationship between measured concentrations of PFOS in serum and potential adverse health effects;
- To understand the kinetics of increases in body burden of PFOS as reflected in serum PFOS measurements;
- To determine if a critical cumulative body burden exists and how this is rleated to toxicokinetics;
- To define the metabolic relationship of PFOS to other Perfluorooctanesulfonyl-based chemicals;
- To understand the primary mechanism of toxicity responsible for early toxic effects;

- To establish reasonable and substantiated no significant risk levels for risk assessment.

Studies initiated in 1998 with PFOS and N-EtFOSE are identified in Tables VI.1 and VI.2, respectively. In addition to these, a 90-day dietary study with N-MeFOSE has been completed and studies have been undertaken to better understand the potential for metabolic degradation of perfluorooctanesulfonamide-based chemicals to PFOS. Descriptive summaries of many of these studies are included in the Appendix.

Table VI.1: PFOS Toxicity Studies Initiated in 1998

Study Title	Start Date	Completion Date (In-Life)
Four-Week Capsule Range-Finder Study in Cynomolgus Monkeys	4/23/98	5/22/98
Six-Month Capsule Feeding Study in Cynomolgus Monkeys	8/5/98	2/8/99 (histo.) 5/7/99 (recov.)
Two-Year Dietary Study in Rats	5/20/98	4/24/2000
Hepatic Peroxisome Induction and Cell Proliferation Study	12/1/98	1/7/98
Biochemical and Molecular Biology Mechanistic Studies	7/1/97	On-going
Segment II Teratology in Rabbits	8/28/98	9/29/98
Two-Generation Reproduction Study in Rats	5/26/98	12/31/98
One-Generation Cross-Fostering Reproduction Study in Rats	11/1/98	2/15/99
One-Generation Reproduction PK Study in Rats (through Gestation)	11/7/98	1/30/99
One-Generation Reproduction PK Study in Rats (through Lactation)	11/23/98	3/1/99

Table VI.2: N-EtFOSE Toxicity Studies Initiated in 1998

Study Title	Start Date	Completion Date (In-Life)
Two-Year Dietary Study in Rats	1/26/98	1/25/2000
Single-Dose ADME in Cynomolgus Monkeys	5/14/98	10/1/98
Biochemical and Molecular Biology Mechanistic Studies	7/1/97	On-going
Segment II Teratology in Rats	8/11/98	9/4/98
Segment II Teratology in Rabbits	8/28/98	9/29/98
Two-Generation Reproduction Study in Rats	6/8/98	12/31/98

104-week dietary carcinogenicity study with perfluorooctane sulfonic acid, potassium salt in rats. In-life Start date: 4/20/98; In-life completion date: 4/24/2000

The purpose of this two year assay is to determine: carcinogenic potential, bioaccumulation with repeated doses, threshold effect levels, a no observable adverse effect level (NOAEL), and mechanisms of toxicity. Proliferating cell nuclear antigen (PCNA) will be measured at early time points in both liver and pancreas as an indicator of preneoplastic lesions. There are six dose groups: 0, 0.5, 2.0, 5.0, 20 and 20 (recovery) ppm PFOS in the feed. Interim sacrifices at weeks 4, 14 and 52 will be performed to determine PCNA, palmitoyl Co-A oxidation, clinical chemistry, and histopathology. Animals in group 6 will be treated for 52 weeks, then treatment will be discontinued and the animals will be observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 26 weeks post-treatment.

Serum samples will be collected for PFOS detection from the scheduled sacrifices and in-life blood draws. Hematology, serum chemistry, urinalysis, urine chemistry, and serum sampling (In-life blood draws) will be done at weeks 14, 27 and 53.

104 -Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats. In-life Start Date: 1/ 26/1998.

The purpose of this study is to assess the carcinogenicity of the test material, N-ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE) when administered in the diet to rats for at least 104 weeks. The test material was administered at dose levels of 0, 1, 3, 30, 100, and 300 ppm. Necropsies were performed during Week 4 and during Week 14 for hepatocellular proliferation rate measurements, hepatic palmitoyl-CoA oxidase activity, and serum and liver PFOS levels. Due to the morbidity and morbundity, the 300 ppm group was terminated at 8 weeks. During Week 8 and 14, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry. At necropsy, macroscopic observations were recorded, organ

weights were obtained, and tissues were placed in fixative as specified by the protocol. Microscopic examinations were done on selected tissues from animals necropsied during Weeks 8 and 14. A recovery group of animals in the 100 ppm dose group will be treated for at least 52 weeks then treatment will be discontinued, and the animals will be observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 26 week post-treatment.

Six-Month Capsule Feeding Study in Cynomolgus Monkeys. In-life Start Date: 8/5/1998. In-life completion date: 5/7/1999.

The purpose of this study is to establish a no observable effect level for PFOS in the Cynomolgus monkey. PFOS is a known hepatic peroxisome proliferator (PP) in the rat. Non-human primates such as the Cynomolgus monkey respond similarly to humans with no to low hepatic response to peroxisome proliferators, and therefore are the appropriate human surrogate species. Blood hormone levels will be determined pretreatment and after 30, 60, 90, and 180 days of treatment, and after 30, 60, and 90 days of recovery. Samples will be analyzed for estradiol, estrone, estriol, thyroid stimulating hormone, and triiodothryonine (T3), thyroxin (T4), cortisol and testosterone. Serum and liver PFOS levels will be determined and correlated to blood chemistries. Urine and feces PFOS determinations will be made day 0 of recovery and after 6, 30, and 90 days of recovery to track elimination kinetics. Four groups of four male and four female Cynomolgus monkeys will be orally dosed with PFOS triturated in lactose at 0, 0.03, 0.15 and 0.75 mg/kg/day daily by gelatin capsule. Dose levels were determined in a previously conducted rangefinder study. Two additional animals in the control, mid and high dose groups will be designated as recovery animals for which treatment will be discontinued at 26 weeks, and the animals will be observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 13 weeks post-treatment. Hematology and clinical chemistry will be determined once before initiation, after 30, 60, 90, and 180 days of treatment, and after 30, 60, and 90 days of recovery. Clinical chemistry will include urea nitrogen, cholesterol, triglicerides, alkaline phosphatase, alanine

aminotransferase, bile acids, total and direct bilirubin, lipase, and pancreatic-specific amylase. Palmitoyl CoA oxidase activity will be analyzed from liver tissue to determine peroxisome proliferation. Complete necropsy will be performed after at least 26 weeks of treatment, and after at least 13 weeks post treatment for the recovery groups. Histopathology and proliferative cell nuclear antigen (PCNA) will be performed on a subset of the specimens.

Metabolism of N-EtFOSE, in Cynomolgus Monkeys following administration of a single dose by Oral Gavage. In-life start date: 5/14/98. In-life end date: 10/1/98

The purpose of this study is to determine the absorption, distribution, metabolism and excretion (ADME) of N-Et FOSE and its metabolites. Determination of ADME parameters in non-human primate is an appropriate model for human risk assessment, as opposed to the rat. Two groups of Cynomolgus monkeys will be used. Group A will have five animals/sex and will be given 10 mg/kg single treatment by oral gavage. Tissues will be collected from one animal per sex per time-point at 0.5, 1, 7, 14 and 28 days. Urine and feces will be collected at 0.5, 1, and daily up to 28 days. Group B will have one animal/sex which will be bile duct cannulated and will be given 10 mg/kg single treatment by oral gavage. Bile will be collected at 0.5, 1, and daily up to 28 days. Blood samples will be drawn for serum (and/or plasma) at pre-dose, 1, 2, 4, 6, 12, 24, 36, 48 hours, 7 days and 14, 21 and 28days for both groups. Tissues with mass > 0.5 g will be analyzed for N-EtFOSE and its metabolites: Cerebrospinal fluid, skin, fat, urinary bladder, testis, epididymus, seminal vesicles, prostate, uterus, spleen, kidneys, liver, thymus, heart, lungs, diaphragm, salivary glands, trachea, esophagus, muscle, bone, bone marrow, pancreas, lymph nodes, tongue, eyes, brain, spinal cord, stomach, small intestine, large intestine, thyroid/parathyroid, aorta (~0.5 g), adrenal glands, gallbladder , and ovaries will be included.

Mono N-EtFOSE Phosphate – Absorption, Disposition, Metabolism, Excretion in Rats. In-life Start Date: 5/15/1998. In-life completion date: 6/15/1998.

The purpose of this study was to assess the potential for absorption and metabolism of the monophosphate ester of FC-807 in rats. FC-807 is the brand name of a fluorochemical based product sold by 3M and approved by the FDA for use as an oil and water repellant in paper and paperboard food packaging. The primary concern of this study was to determine if and to what extent the monoester is absorbed from the intestinal tract, and to what extent it can be metabolized ton N- ethyl (perfluorooctane)sulfonamido ethanol (N-Ethyl FOSE) and other metabolites, including PFOS, once in the blood stream. Both a time-course study following a single dose of compound, and a dose response study were conducted. In the time course study, rats were dosed orally, via gavage, or by intravenous (i.v.) injection, via the tail vein, with FC-807 monoester. One animal from each dosage route group was sacrificed two hours after dosing. Urine and feces were collected daily for four days. On day 4, one animal from each group was sacrificed, and the remainder of the animals were sacrificed 28 days post dose. The preliminary results indicated absorption of the oral 50 mg/kg dose and metabolism at day 4, and day 28. The i.v doses of 0.5 mg/kg were also extensively metabolized and were deemed to be too high. The dose-response study consisted of six dose groups. There was one control group, three groups dosed orally at 0.01, 0.1, and 5 mg/kg, via gavage, and two groups that received tail vein i.v. injections at 0.01, and 0.1 mg/kg at a volume of 5 ml/kg on day one of the study to each group. All groups were sacrificed on day 4 of the study.

13-week Dietary Toxicity Study with N-Methyl FOSE in Rats. In-life Start Date: 9/1/1998. In life completion date: 12//8/1998.

The purpose of this study was to develop sub-chronic toxicity data and the toxicokinetics for N-Methyl FOSE when administered in the diet to rats for at least 13 weeks. The study design included 20 males and 20 female animals/group fed 0, 3,

30 and 100 ppm N-MeFOSE in the diet. After 4 weeks of treatment, 5 rats/sex/group were sacrificed for Palmitoyl CoA oxidase, PCNA, liver and sera PFOS analysis.

Two-Generation Rat Reproduction Study with PFOS. In-life Start Date: 5/26/1998. In-life completion date: 12/31/1998.

The purpose of this study is to test for toxic effects of PFOS on reproductive function of dosed male and female rats and to assess whether rat pups exposed to the compound in utero & via milk have any developmental, learning and reproductive effects. A secondary objective will be to assess compound and/or metabolite levels in serum and liver from parent animals. Groups of 35 rats will be dosed daily via gastric gavage at dose levels of 0, 0.1, 0.4, 1.6 and 3.2 mg/kg/d. Dosing will start 4 weeks prior to mating, will continue during mating, and for females will continue during gestation and lactation. Reproductive performance will be assessed by fertility index, gestation index, number of pups/ litter, pup viability index and lactation index. The F1 generation pups at each dose level will be assessed for developmental objectives, undergo learning testing, and reproductive performance. Serum and liver samples will be collected at necropsy from five male and five female rats/dose group from F_0 (Parent) animals and analyzed for compound by 3M.

Two-Generation Rat Reproduction Study with N-EtFOSE. Start Date: 6/8/1998. In life completion date: 12/31/1998.

The purpose of this study is to test for toxic effects of N-EtFOSE on reproductive function of dosed male and female rats and to assess whether rat pups exposed to the compound in utero & via milk have any developmental, learning and reproductive effects. A secondary objective will be to assess compound and/or metabolite levels in serum and liver from parent animals. Groups of 35 rats will be dosed daily via gastric gavage at dose levels of 0, 1, 5, 10 and 15 mg/kg/d. Dosing will start six weeks prior to mating, will continue during mating, and for females will continue during gestation and lactation. Reproductive performance will be assessed by fertility index, gestation index, number of pups/ litter, pup viability index and lactation index. The F1

generation pups at each dose level will be assessed for developmental objectives, undergo learning testing and reproductive performance. Serum and liver samples will be collected at necropsy from 5 male and 5 female rats/dose group from F_o (Parent) animals and analyzed for compound by 3M.

Segment II Teratology in Rabbits with PFOS. In-life Start date: 8/28/1998. In-life end date 9/29/1998.

The purpose of this study is to detect adverse effects of PFOS on New Zealand White pregnant female rabbits and development of the embryo on fetus consequent to exposure of the doe from implantation to closure of the hard palate. Dose groups of 22 presumed pregnant female rabbits were dosed with 0, 0.1, 2.0, 2.5 and 3.75 mg/kg/day via a stomach tube. A toxicokinetic satellite group of female rabbits (5 at the control and high dose levels plus three at the other dose levels) were sacrificed at day 21 of gestation (the day following the last dosage) serum, liver, fetal and placental samples were analyzed for PFOS and possible metabolites. Rabbits were Caesarian sectioned on day 29 of presumed gestation. The fetuses were examined for body weight, gross alterations and skeletal alterations, number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions.

Segment II Teratology with N-EtFOSE in rats. In-life Start date: 9/4/1998. In life end date 9/11/1998.

The purpose of this study was to detect adverse effects of N-EtFOSE on presumed pregnant female rats and development of the embryo and fetus consequent to exposure of the dam from implantation to closure of the hard palate. Dose groups of 25 presumed pregnant female rats were dosed with 0, 1, 5, 10 and 20 mg/kg/day by oral gavage. A toxicokinetic satellite group of female rats (five at the control and high dose levels plus three at the other dose levels) were sacrificed at day 18 of gestation (the day following the last dosage) serum, liver, fetal and placental samples were analyzed for N-EtFOSE and possible metabolites. Rats were Caesarian

sectioned on day 20 of presumed gestation. The fetuses were examined for body weight, gross alterations and skeletal alterations, number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions.

Segment II Teratology in Rabbits with N-EtFOSE. In-life Start date: 8/28/1998. In life end date 9/29/1998.

The purpose of this study was to detect adverse effects of N-EtFOSE on New Zealand White pregnant female rabbits and development of the embryo and fetus consequent to exposure of the doe from implantation to closure of the hard palate. Dose groups of 22 presumed pregnant female rabbits were dosed with 0, 0.1, 2.0, 2.5 and 3.75 mg/kg/day via a stomach tube. A toxicokinetic satellite group of female rabbits (five at the control and high dose levels plus three at the other dose levels) were sacrificed at day 21 of gestation (the day following the last dosage) serum, liver, fetal and placental samples were analyzed for N-EtFOSE and possible metabolites. Rabbits were Caesarian section on day 29 of presumed gestation. The fetuses will be examined for body weight, gross alterations and skeletal alterations, number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions.

Current and Proposed Human Health and Exposure Research Initiatives

At present, there are several ongoing research studies and proposals. These are briefly outlined below.

1. Half-life Fluorochemical Determination Study. 3M retirees from the Decatur and Cottage Grove plants will be asked to participate in a study to determine the half-life of PFOA and PFOS. Retirees' serum will be analyzed semi-annually for the next five years for PFOS, PFOA and perfluorohexane sulfonate.

2. Decatur Serum Exposure Assessment Study. Employees at the 3M Decatur chemical plant have in the past voluntarily participated in a fluorochemical medical surveillance program. Analysis of the surveillance data has not shown significant associations between the employees' clinical chemistry and hematology tests and either total serum organic fluorine or serum PFOS levels. However, the voluntary nature of the medical surveillance program does not allow for a complete understanding of the distribution of employee fluorochemical serum levels. In order to address this issue, a random sample of approximately 80 Decatur film plant and 125 Decatur chemical plant employees will be asked to participate in a serum determination study for the following fluorochemicals: perfluorooctane sulfonate, perfluorooctane sulfonate amide, perfluorohexane sulfonate, perfluorooctanoic acid, N-ethyle perfluorooctnae sulfonamido ethanol and its acetate derivitive, and N-methyl perfluoroctanesulfonamido ethanol. A sub-sample of employees will also be tested for total serum organic fluorine. A brief questionnaire will also be administered to each employee inquiring about current and past work history as well as possible routes of oral ingestion.

3. An Epidemiologic Analysis of the Inpatient Claims Experience of 3M Decatur Employees, 1993-1997. The purpose of this study is to examine the inpatient claims database, as maintained by Corporate Health Strategies, from January 1, 1993 – December 31, 1997 of Decatur plant employees. The data will be stratified by whether the employees are in the chemical or film plants. The study population will include full-time

active employees who worked throughout the five year interval, all full-time active employees who quit, died or went on long-term disability within the five year interval, and all employees who have retired. Observed inpatient claims for each plant population will be compared to an expected experience based on the 3M normative database. Inpatient claims analysis will proceed in a sequential, hierarchical manner. First we will examine Medical Diagnosis Codes; next Diagnosis Related Groups, and finally selected ICD-9 codes within each DRG.

4. PFOS and PFOA Retrospective Cohort Mortality Studies of Employees at the 3M Decatur and Cottage Grove Plants. Previous retrospective cohort mortality studies have been conducted at the Decatur and Cottage Grove plants. However, neither of these studies utilized the employees' fluorochemical serum measurements to design job-, department- and calendar-year exposure matrices. The purpose of these two studies is to construct PFOS and PFOA exposure matrices based on previously collected employee serum PFOS and PFOA measurements in conjunction with their plant work history experiences. Person-years will be allocated based on the exposure matrices to calculate the traditional measures of risk, i.e., Standardized Mortality Ratios, for more than 50 causes of death. Vital status and cause of death will be ascertained through December 31, 1997.

5. Geographical Reliability Study. The purpose of this study is to determine whether there are geographical differences in human serum measurements in the United States. We will request serum samples from the 18 blood banks that originally participated in the fluorochemical determination study (see Table II.3) to determine whether comparable serum levels are measured.

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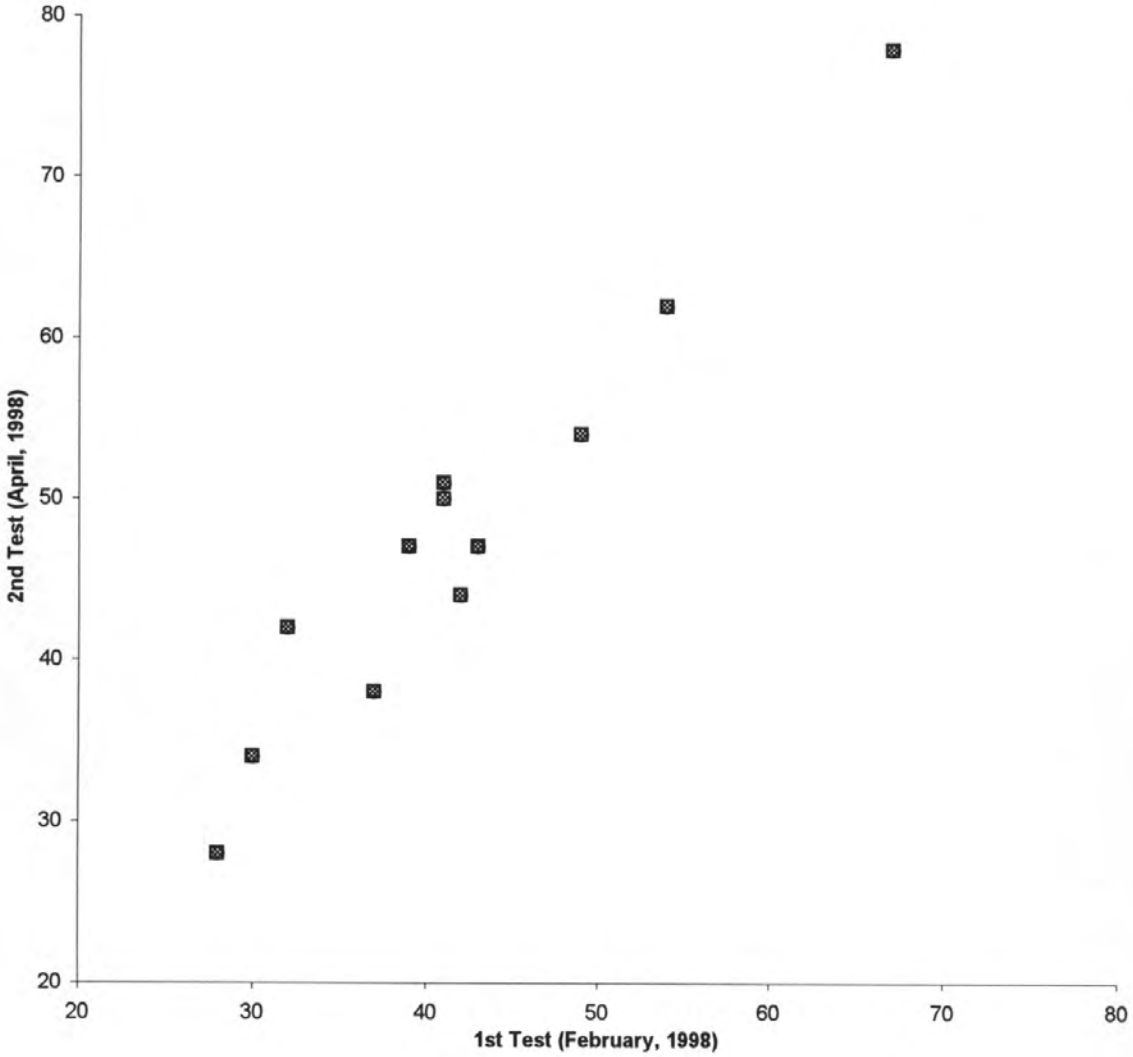
APPENDIX

FIGURE A1. Reliability of PFOS Serum Measurements in 12 3M Employees



Appendix – Table A1

Mean, Median, Standard Deviation (SD) of Mean and Range of PFOS, Demographic, Serum Chemistries and Hematological for Antwerp and Decatur Employees Combined, 1995 (N=178) and 1997 (N=147)

PFOS* (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
				PFOS (ppm)				
0 - < 1ppm	0.49[1]	0.5	0.27	000 - 0.90	0.52[1]	0.52	0.27	0.10 - 0.97
1 - < 3ppm	1.82[1]	1.77	0.58	1.00 - 2.91	1.78[1]	1.64	0.56	1.02 - 2.89
3 - < 6ppm	4.12[1]	3.97	0.81	3.00 - 5.80	3.87[1]	3.59	0.70	3.09 - 5.30
≥ 6ppm	8.17[1]	7.73	2.52	6.06 - 12.83	7.20[1]	6.68	1.59	6.05 - 9.3
	F value = 321.9, p < .0001				F value = 367.6, p < .0001			
				Age (yrs)				
0 - < 1 ppm	37	36	8	21 - 58	36	34	11	21 - 62
1 - < 3 ppm	42[2]	41	9	25 - 60	42[2]	41	9	24 - 63
3 - < 6 ppm	40	40	7	26 - 55	41	42	5	32 - 54
≥ 6 ppm	45	43	7	37 - 56	42	45	9	29 - 52
	F value = 3.7, p = .02				F value = 5.1, p = .002			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
Alcohol (drinks/day)								
0 - < 1ppm	0.8	0.6	0.9	0.0 - 3.6	0.5	0.1	0.8	0 - 4.3
1 - < 3 ppm	0.5	0.1	0.7	0.0 - 3.6	0.5	0.1	0.8	0 - 5.0
3 - < 6 ppm	1.2	0.3	1.9	0.0 - 6.0	1.0	0.1	1.8	0 - 7.1
≥ 6ppm	0.7	0.0	1.1	0.0 - 2.9	0.2	0.1	0.2	0.1 - 0.8
	F value = 4.0, p = .009				F value = 1.8, p = .15			
BMI (kg/m^2)								
0 - < 1 ppm	25.5	24.8	4.2	17.9 - 38.7	26.0	24.9	4.9	20.1 - 41.7
1 - < 3 ppm	27.7	26.3	5.8	19.6 - 60.7	27.7	26.4	5.7	18.1 - 48.5
3 - < 6 ppm	24.9[3]	25.0	3.8	17.9 - 32.5	27.3	27.9	4.4	19.1 - 36.0
≥ 6 ppm	27.7	29.4	4.2	20.6 - 33.0	30.8	29.7	4.0	26.1 - 36.2
	F value = 3.7, p = .02				F value = 2.1, p = .10			
Cigarettes (per day)								
0 - < 1 ppm	2.6	0.0	6.4	0.0 - 25.0	4.7	0.0	9.4	0 - 40
1 - < 3 ppm	6.8	0.0	11.3	0.0 - 40.0	8.2	0.0	11.3	0 - 40
3 - < 6 ppm	10.6	8.0	12.4	0.0 - 40.0	4.1	0.0	8.3	0 - 30
≥ 6 ppm	0.4	0.0	1.1	0.0 - 3.0	6.0	0.0	13.4	0 - 30
	F value = 4.8, p = .003				F value = 1.5, p = .23			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				BUN				
0 - < 1ppm	16.5	15	3.5	11 - 26	14.5	14.0	2.8	9.0 - 21.0
1 - <3 ppm	15.4	15.0	4.0	8.0 - 26.0	14.2	14.0	3.2	6.0 - 26.0
3 - < 6 ppm	16.4	16.0	3.7	10.0 - 23.0	15.0	15.0	2.9	9.0 - 20.0
≥ 6 ppm	15.1	14.0	4.2	10.0 - 21.0	13.8	12.0	4.1	9.0 - 19.0
	F value = 1.1, p = .36				F value = 0.5, p = 0.67			
				Creatinine				
0 - < 1ppm	1.0	1.0	0.2	0.7 - 1.6	0.9	0.9	0.1	0.6 - 1.2
1 - <3 ppm	1.0	1.0	0.2	0.7 - 1.6	0.9	0.9	0.1	0.7 - 1.3
3 - < 6 ppm	0.9	0.9	0.2	0.7 - 1.2	0.9	0.9	0.1	0.7 - 1.1
≥ 6 ppm	1.1	1.2	0.3	0.6 - 1.6	1.0	0.9	0.2	0.8 - 1.4
	F value = 2.3, p = .08				F value = 0.4, p = 0.78			
				Glucose				
0 - < 1 ppm	85	85	15	66 - 170	87	85	17	58 - 174
1 - <3 ppm	86	86	22	60 - 260	93	84	39	65 - 303
3 - < 6 ppm	84	83	12	66 - 114	95	89	25	74 - 192
≥ 6 ppm	84	83	14	71 - 105	89	88	7	80 - 97
	F value = 0.9, p = .44				F value = 0.6, p = .59			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				Alkaline Phosphatase				
0 - < 1 ppm	80	78	22	31 - 158	77	73	17	49 - 132
1 - <3 ppm	89	89	27	49 - 191	83	79	23	41 - 163
3 - < 6 ppm	86	85	21	32 - 124	76	74	22	29 - 120
≥ 6 ppm	88	85	24	63 - 136	88	84	18	65 - 114
	F value = 1.3, p = .28				F value = 1.17, p = .32			
				GGT				
0 - < 1 ppm	43	31	28	16 - 155	28	22	20	10 - 142
1 - <3 ppm	47	36	39	2 - 293	36	25	33	10 - 179
3 - < 6 ppm	40	39	15	21 - 80	28	27	14	13 - 71
≥ 6 ppm	43	33	18	28 - 79	33	37	12	17 - 48
	F value = 0.5, p = .71				F value = 1.1, p = .34			
				AST				
0 - < 1 ppm	27	25	13	15 - 96	27	25	7	13 - 53
1 - <3 ppm	29	26	12	14 - 90	26	25	7	15 - 56
3 - < 6 ppm	25	24	5	13 - 37	25	23	7	14 - 43
≥ 6 ppm	33	33	6	26 - 43	29	28	3	26 - 34
	F value - 1.8, p = .14				F value = 0.5, p = .67			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				ALT				
0 - < 1 ppm	48	43	20	27 - 118	31	30	11	13 - 60
1 - <3 ppm	46	42	21	18 - 183	33	29	16	10 - 89
3 - < 6 ppm	42	41	7	30 - 59	34	31	18	14 - 82
≥ 6 ppm	51	49	17	29 - 82	41	45	10	25 - 49
	F value = 1.0, p = .38				F value = 0.9, p = .46			
				Total Bilirubin				
0 - < 1 ppm	0.88	0.70	0.50	0.40 - 2.90	0.77	0.60	0.40	0.30 - 2.30
1 - <3 ppm	0.66[2]	0.60	0.30	0.20 - 1.50	0.61[2]	0.60	0.21	0.30 - 1.30
3 - < 6 ppm	0.64[2]	0.60	0.28	0.20 - 1.40	0.63	0.50	0.31	0.40 - 1.30
≥ 6 ppm	0.76	0.70	0.23	0.50 - 1.20	0.58	0.50	0.24	0.40 - 1.00
	F value = 4.4, p = .005				F value = 2.9, p = .04			
				Direct Bilirubin				
0 - < 1 ppm	0.22	0.20	0.05	0.02 - 0.40	0.15	0.10	0.07	0.10 - 0.40
1 - <3 ppm	0.21	0.20	0.06	0.10 - 0.40	0.12[2]	0.10	0.04	0.10 - 0.20
3 - < 6 ppm	0.21	0.20	0.04	0.20 - 0.30	0.12	0.10	0.04	0.10 - 0.20
≥ 6 ppm	0.20	0.20	0.02	0.10 0.30	0.10	0.10	0.00	0.10 - 0.10
	F value = 0.6, p = .58				F value = 3.5, p = .02			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				Cholesterol				
0 - <1 ppm	219	215	47	100 - 340	198	197	40	110 - 280
1 - <3 ppm	216	213	43	118 - 315	216	219	42	116 - 365
3 - <6 ppm	214	214	35	128 - 278	229[2]	224	29	192 - 321
≥ 6 ppm	213	221	36	160 - 251	229	238	26	186 - 250
	F value = 0.1, p =.96				F value = 4.3, p =.006			
				LDL				
0 - <1 ppm	140	137	43	29 - 261	124	128	34	50 - 205
1 - < 3 ppm	134	134	40	44 - 234	141	134	38	61 - 290
3 - <6 ppm	137	135	34	65 - 190	148[2]	142	24	111 - 196
≥ 6 ppm	142	136	32	95 - 178	145	156	26	103 - 164
	F value = 0.2 , p =.87				F value = 3.7, p =.01			
				HDL				
0 - <1 ppm	53	53	13	31 - 94	46	48	11	19 - 74
1- <3 ppm	48	47	13	26 -94	44	45	10	28 - 69
3 - <6 ppm	45	46	11	23 - 74	48	47	10	32 - 69
≥ 6 ppm	45	46	9	34 - 61	40	38	4	37 - 45
	F value = 2.9, p =.04				F value = 1.1, p =.34			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				Triglycerides				
0 - <1 ppm	129	96	98	41 - 622	148	107	162	38 - 1209
1- <3 ppm	161	133	107	41 - 651	156	122	108	44 - 534
3 - <6 ppm	158	142	88	34 - 413	166	158	92	45 - 394
≥ 6 ppm	132	151	45	64 - 187	220	191	83	149 - 352
	F value = 1.1, p =.35				F value = 0.5, p =.67			
				Hematocrit				
0 - <1 ppm	47	47	2	43 - 51	46	46	2	40 - 52
1 - <3 ppm	46	46	3	38 - 52	45	46	3	39 - 53
3 - <6 ppm	47	47	2	41 - 52	46	44	3	39 - 50
≥ 6 ppm	47	48	2	44 - 49	45	44	2	42 - 47
	F value = 2.4, p =.07				F value = 2.1, p = .11			
				Hemoglobin				
0 - <1 ppm	15.5	15.5	0.7	14.0 - 16.7	15.5	15.5	0.8	13.5 - 17.0
1 - <3 ppm	15.2	15.2	1.0	13.0 - 17.4	15.4	15.5	0.9	13.3 - 17.3
3 - <6 ppm	15.5	15.5	0.8	13.6 - 17.4	15.0	14.7	1.0	12.5 - 16.7
≥ 6 ppm	15.5	15.4	0.7	14.7 - 16.2	15.1	15.0	0.7	14.1 - 16.2
	F value = 2.2, p =.10				F value = 1.8, p =.15			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				RBC				
0 - <1 ppm	5.0	5.0	0.3	4.3 - 5.7	5.1	5.2	0.3	4.3 - 5.9
1 - <3 ppm	4.9	4.9	0.3	4.3 - 5.7	5.0	5.1	0.3	4.1 - 5.5
3 - <6 ppm	5.0	5.0	0.2	4.6 - 5.5	5.0	5.0	0.3	4.4 - 5.7
≥ 6 ppm	5.0	4.9	0.5	4.0 - 5.7	5.0	4.9	0.3	4.7 - 5.5
	F value = 0.4, p = .75				F value = 1.4, p = .25			
				MCH				
0 - <1 ppm	31.3	31.2	1.6	27.7 - 34.3	30.4	30.3	1.5	27.6 - 34.1
1 - <3 ppm	30.9	30.9	1.4	26.4 - 34.3	30.6	30.5	1.2	26.7 - 33.8
3 - <6 ppm	31.2	31.3	1.5	26.0 - 33.6	30.2	31.2	1.9	26.2 - 32.9
≥ 6 ppm	31.2	30.5	2.8	28.2 - 36.9	30.5	30.5	2.1	27.5 - 33.4
	F value = 0.9 , p =.45				F value = 0.6, p = .65			
				MCHC				
0 - <1 ppm	33.1	33.2	0.7	31.9 - 34.7	33.6	33.6	0.5	32.2 - 34.9
1 - <3 ppm	33.2	33.1	0.6	31.7 - 34.5	33.6	33.6	0.5	31.8 - 34.6
3 - <6 ppm	33.1	32.6	0.7	31.3 - 34.3	33.5	33.5	0.7	32.0 - 34.4
≥ 6 ppm	33.1	33.3	0.6	32.2 - 34.0	33.9	33.9	0.5	33.4 - 34.6
	F value = 0.2, p = .90				F value = 0.7, p = .56			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				MCV				
0 - <1 ppm	94	95	5.2	85-106	90	90	4.4	83 – 101
1 - <3 ppm	93	94	4.3	80-104	91	91	3.6	81 – 99
3 - <6 ppm	94	94	4.8	81-104	90	91	5.2	80 – 97
≥ 6 ppm	94	92	9.7	85-115	90	91	5.7	81 – 96
	F value = 1.1, p = .35				F value = 0.6, p = .59			
				WBC				
0 - <1 ppm	6.1	6.0	1.3	4.1 – 9.4	6.1	5.8	1.4	3.8 – 10.3
1 - <3 ppm	7.0[2]	6.8	2.0	3.6 – 15.5	6.9	6.6	1.9	3.8 – 13.2
3 - <6 ppm	7.6[2]	6.9	2.2	4.1 – 13.3	6.2	6.1	1.5	4.0 – 10.0
≥ 6 ppm	7.0	6.9	0.6	6.4 – 7.8	6.2	7.1	1.5	4.2 – 7.4
	F value = 4.3, p =.006				F value = 2.2, p =.09			
				Platelets				
0 - <1 ppm	226	224	40	159 – 309	227	220	53	106 – 406
1 - <3 ppm	229	224	47	122 – 367	229	223	49	124 – 359
3 - <6 ppm	228	226	45	132 – 344	220	221	45	147 – 316
≥ 6 ppm	185	182	50	132 – 277	199	191	58	146 – 295
	F value = 2.1, p =.10				F value = 0.6, p = 0.60			

Table A1 (continued)

1. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of the other PFOS categories.
2. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of 0 - < 1 ppm PFOS category.
3. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of 1 - < 3 ppm category.

* Sample Size	1995 Data	1997 Data
0 - <1 ppm	45	60
1 - <3 ppm	91	63
3 - <6 ppm	35	21
≥ 6 ppm	7	5

Table A2. Description of Final Employment and Vital Status for Male (n = 1,639) and Female (n = 318) Employees

Final Employment Status and Vital Status	Male Employees N	Male Employees %	Female Employees N	Female Employees %
Currently Employed	810	49.4	141	44.3
Retired				
Alive	59	3.6	9	2.8
Deceased	10	0.6	0	0.0
Unknown	0	0.0	0	0.0
Terminated				
Alive	694	42.3	164	51.6
Deceased	29	1.8	1	0.3
Unknown	6	0.4	0	0.0
Died While Employed	31	1.9	3	0.9
TOTAL	1,639	100.0*	318	100.0*

*percentages may not add to 100 due to rounding

Table A3. Characteristics of Male (n = 1,639) and Female (n = 318) Employees

Variable	Males	Females
Number of Employees 1,639	318	
Number of Person-years	33,108	4,807
Number of Deaths	70	4
Average Age Started Work	25	26
Average Year of Entry	1971	1977
Average Age at Death	47	28
Average Year of Death	1984	1980

Table A4. Distribution of Men
by Age and Year of Entry into Follow-up (n = 1,639)

	Year of Entry into Follow-up							
Age	1960 to 1964	1965 to 1969	1970 to 1974	1975 to 1979	1980 to 1984	1985 to 1989	1990 to 1991	TOTAL
<20	18	34	86	31	4	2	0	175
20-24	114	238	301	96	28	18	3	798
25-29	64	139	65	37	21	14	5	345
30-34	21	58	33	19	8	10	4	153
35-39	22	25	9	15	6	5	1	83
40-44	19	18	4	10	8	2	0	61
45-49	3	3	4	5	0	2	0	17
50-54	0	1	0	0	2	3	0	6
55-59	0	1	0	0	0	0	0	1
60+	0	0	0	0	0	0	0	0
TOTAL	261	517	502	213	77	56	13	1,639

Table A5. Distribution of Women
by Age and Year of Entry into Follow-up (n = 318)

	Year of Entry into Follow-up							
Age	1960 to 1964	1965 to 1969	1970 to 1974	1975 to 1979	1980 to 1984	1985 to 1989	1990 to 1991	TOTAL
<20	2	12	18	16	4	0	0	52
20-24	4	22	23	50	4	9	1	113
25-29	3	4	4	47	4	5	3	70
30-34	2	3	2	15	1	4	2	29
35-39	1	2	3	16	3	3	2	30
40-44	0	0	0	8	3	2	0	13
45-49	0	0	0	5	1	1	0	7
50-54	0	0	0	3	0	0	0	3
55-59	0	0	0	1	0	0	0	1
60+	0	0	0	0	0	0	0	0
TOTAL	12	43	50	161	20	24	8	318

Table A6. Cause-Specific SMRs for Men
Ever Employed in Chemical Department (s)
Using the U.S. as Comparison Population (n = 1,050)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
All Causes of Death	57	81.5	70.0	(53.0, 90.6)
All Malignant Neoplasms	13	16.9	76.9	(40.9, 131.5)
Cancer of Buccal Cavity and Pharynx	0	0.5	---	(0.0, 800.6)
Cancer of Digestive Organs and Peritoneum	1	3.6	27.5	(0.7, 153.2)
Cancer of Esophagus	0	0.4	---	(0.0, 913.3)
Cancer of Stomach	0	0.5	---	(0.0, 686.1)
Cancer of Large Intestine	1	1.3	76.9	(1.9, 428.5)
Cancer of Rectum	0	0.3	---	(0.0, 1,271.9)
Cancer of Liver and Biliary Passages	0	0.3	---	(0.0, 1,188.2)
Cancer of Pancreas	0	0.8	---	(0.0, 480.6)
Cancer of All Other Digestive Organs	0	0.1	---	(0.0, 3,203.2)
Cancer of Respiratory System	7	6.1	115.1	(46.3, 237.1)
Cancer of Larynx	0	0.2	---	(0.0, 1,891.9)
Cancer of Bronchus Trachea and Lung	7	5.8	120.7	(48.5, 248.7)
Cancer of Other Respiratory Organs	0	0.1	---	(0.0, 4,214.1)
Cancer of Prostate	0	0.5	---	(0.0, 805.9)
Cancer of Testes and Other Male Genital Organs	0	0.2	---	(0.0, 1,678.5)
Cancer of Kidney	0	0.5	---	(0.0, 768.2)
Cancer of Bladder and Other Urinary Organs	1	0.2	415.5	(10.4, 2,315.3)

Table A6. (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
Cancer of Brain and Other Central Nervous System	1	0.9	117.2	(2.9, 653.0)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	---	(0.0, 5,113.2)
Cancer of Bone	0	0.1	---	(0.0, 4,368.3)
Cancer of All Lymphatic and Hematopoietic Tissue	2	2.2	92.9	(11.2, 335.5)
Lymphoma	0	0.3	---	(0.0, 1,341.4)
Hodgkin's Disease	0	0.3	---	(0.0, 1,163.3)
Leukemia and Aleukemia	1	0.8	120.0	(3.0, 668.8)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.7	137.2	(3.4, 764.5)
Malignant Melanoma of Skin	0	0.6	---	(0.0, 614.7)
All Other Malignant Neoplasms	1	1.5	68.9	(1.7, 383.7)
Diabetes Mellitus	0	1.1	---	(0.0, 323.9)
Cerebrovascular Disease	1	2.3	43.4	(1.1, 241.7)
All Heart Disease	11	22.5	48.8	(24.4, 87.4)
Hypertension	0	0.1	---	(0.0, 3,389.9)
Respiratory Disease	0	3.2	---	(0.0, 116.3)
Ulcer of Stomach and Duodenum	0	0.2	---	(0.0, 1,796.3)
Cirrhosis of Liver	3	3.0	100.2	(20.7, 292.7)
Nephritis and Nephrosis	0	0.3	---	0.0, 1,308.2)
External Causes	20	22.2	90.2	(55.1, 139.3)
Accidents	16	13.6	117.3	(67.0, 190.5)
Motor Vehicle Accidents	8	7.8	102.8	(44.4, 202.5)
All Other Accidents	8	5.9	135.7	(58.6, 267.4)
Suicides	3	5.2	57.7	(11.9, 168.6)
Homicides and Other External Causes	1	3.2	31.4	(0.8, 175.1)
Residual Causes*	7	9.0	77.6	(31.2, 159.9)
Unknown Causes &	2			

*all other causes of death combined

& no death certificate obtained; included only in all causes of death category

Table A7. Cause-Specific SMRs for Men
Only Employed in Chemical Department (s)
Using the U.S. as Comparison Population (n = 485)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
All Causes of Death	32	44.1	72.5	(49.6, 102.4)
All Malignant Neoplasms	9	9.6	93.6	(42.8, 177.8)
Cancer of Buccal Cavity and Pharynx	0	0.3	---	(0.0, 1,396.4)
Cancer of Digestive Organs and Peritoneum	0	2.1	---	(0.0, 175.2)
Cancer of Esophagus	0	0.2	---	(0.0, 1,577.7)
Cancer of Stomach	0	0.3	---	(0.0, 1,195.2)
Cancer of Large Intestine	0	0.8	---	(0.0, 488.5)
Cancer of Rectum	0	0.2	---	(0.0, 2,175.6)
Cancer of Liver and Biliary Passages	0	0.2	---	(0.0, 2,072.6)
Cancer of Pancreas	0	0.5	---	(0.0, 827.2)
Cancer of All Other Digestive Organs	0	0.1	---	(0.0, 5,692.4)
Cancer of Respiratory System	5	3.6	141.0	(45.8, 329.0)
Cancer of Larynx	0	0.1	---	(0.0, 3,198.4)
Cancer of Bronchus Trachea, and Lung	5	3.4	147.8	(48.0, 344.9)
Cancer of Other Respiratory Organs	0	0.1	---	(0.0, 7,763.4)
Cancer of Prostate	0	0.3	---	(0.0, 1,202.0)
Cancer of Testes and Other Male Genital Organs	0	0.1	---	(0.0, 3,501.4)
Cancer of Kidney	0	0.3	---	(0.0, 1,365.0)
Cancer of Bladder and Other Urinary Organs	1	0.2	669.9	(16.7, 3,732.7)

Table A7. (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
Cancer of Brain and Other Central Nervous System	1	0.5	222.6	(5.6, 1,240.3)
Cancer of Thyroid and Other Endocrine Glands	0	0.0	---	(0.0, 9,544.3)
Cancer of Bone	0	0.0	---	(0.0, 8,697.5)
Cancer of All Lymphatic and Hematopoietic Tissue	2	1.2	174.0	(21.1, 628.7)
Lymphoma	0	0.2	---	(0.0, 2,466.1)
Hodgkin's Disease	0	0.2	---	(0.0, 2,346.1)
Leukemia and Aleukemia	1	0.4	226.3	(5.7, 1,260.7)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.4	249.5	(6.2, 1,390.1)
Malignant Melanoma of Skin	0	0.3	---	(0.0, 1,200.4)
All Other Malignant Neoplasms	0	0.8	---	(0.0, 455.3)
Diabetes Mellitus	0	0.6	---	(0.0, 589.0)
Cerebrovascular Disease	0	1.3	---	(0.0, 274.4)
All Heart Disease	7	13.1	53.4	(21.5, 110.1)
Hypertension	0	0.1	---	(0.0, 5,834.4)
Respiratory	0	1.9	---	(0.0, 195.3)
Ulcer of Stomach and Duodenum	0	0.1	---	(0.0, 3,083.3)
Cirrhosis of Liver	1	1.6	62.3	(1.6, 347.3)
Nephritis and Nephrosis	0	0.2	---	(0.0, 2,346.9)
External Causes	10	10.5	95.0	(45.6, 174.7)
Accidents	9	6.5	139.4	(63.7, 264.5)
Motor Vehicle Accidents	5	3.6	138.0	(44.8, 322.0)
All Other Accidents	4	2.9	140.2	(38.2, 359.0)
Suicides	1	2.5	40.0	(1.0, 223.1)
Homicides and Other External Causes	0	1.5	---	(0.0, 247.4)
Residual Causes*	3	4.7	63.4	(13.1, 185.3)
Unknown Causes &	2			

*all other causes of death combined

& no death certificate obtained; included only in all causes of death category

January 21, 1999

Rat Oral (diet) Carcinogenicity on N-EtFOSE

Study Outline

Study Objectives: Main Objective – To determine the carcinogenicity of N-EtFOSE upon chronic oral administration.

Secondary Objectives – To determine chronic and subchronic toxicity of N-EtFOSE after 52 and 13 weeks, respectively, of compound administration. To determine compound's potential for perixosme proliferation (palmitoyl-CoA oxidase activity) and hepatocyte proliferation (PCNA, proliferative cell nuclear antigen). To measure compound and/or metabolite levels in the liver and serum after various time-points of exposure.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Eight – controls (two groups), 1, 3, 30, 100 & 300 ppm in diet plus a 100 ppm recovery group (receive compound for 52 weeks followed by 52 weeks without compound).

Number per Group: 50 males and 50 females per group plus additional animals for interim sacrifices at 4, 13 & 52 weeks. (Note the 100 ppm recovery group has 40 males and 40 females).

Test Article: N-EtPFOS furnished by Sponsor who is responsible for compound identity and purity.

Compound Administration: Daily administration for up to two-years (104 weeks) admixed in the diet.

Clinical Observations: Twice daily.

Body Weights: Once a week for the first 16 weeks; once every four weeks thereafter.

Food Consumption: Once a week for the first 16 weeks; once every four weeks thereafter.

Hematology and Clinical Chemistry: Ten rats/sex from 0, 1, 3, 30 & 100 ppm groups at weeks 14, 52 and 104.

Urinalysis: Ten rats/sex from 0, 1, 3, 30 & 100 ppm groups at weeks 14, 52 and 104.

Interim Necropsies: Five rats/sex from 0, 1, 3, 30, 100 & 300 ppm groups at 4 and 14 weeks for PCNA, palmitoyl CoA oxidase, and compound level samples. (Note samples for organ weights and histopathology taken at 14 weeks.) Ten rats/sex from 0, 1, 3, 30 &100 ppm groups at 52 weeks for compound levels, organ weight, histopathology samples.

Organ Weights: Measured at 14 and 52 week interim necropsies.

Histopathology: Microscopic examination of selected tissues at 14 and 52 week animals. Complete tissue examination of 0 (one control group), 3, 30, and 100 ppm 104 weeks animals. Selected tissue examination of 100 ppm recovery animals. Histopathologic results will indicate extend of tissue examination of 1 ppm and second control group.

Study Monitor: Andrew Seacat; Alternate, Marv Case

January 21, 1999

Rat Oral (diet) Carcinogenicity on PFOS

Study Outline

Study Objectives: Main Objective – To determine the carcinogenicity of PFOS upon chronic oral administration.

Secondary Objectives – To determine chronic and subchronic toxicity of after 52 and 13 weeks, respectively, of compound administration. To determine compound's potential for perixosme proliferation (palmitoyl-CoA oxidase activity) and hepatocyte proliferation (PCNA, proliferative cell nuclear antigen). To measure compound and/or metabolite levels in the liver and serum after various time-points of exposure.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Six – 0, 0.5, 2, 5, & 20 ppm in diet plus a 20 ppm recovery group (receive compound for 52 weeks followed by 52 weeks without compound).

Number per Group: 50 males and 50 females per group plus additional animals for interim sacrifices at 4, 13 & 52 weeks. (Note the 20 ppm recovery group has 40 males and 40 females).

Test Article: PFOS furnished by Sponsor who is responsible for compound identity and purity.

Compound Administration: Daily administration for up to two-years (104 weeks) admixed in the diet.

Clinical Observations: Twice daily.

Body Weights: Once a week for the first 16 weeks; once every four weeks thereafter.

Food Consumption: Once a week for the first 16 weeks; once every four weeks thereafter.

Hematology and Clinical Chemistry: Ten rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at weeks 14, 52 and 104.

Urinalysis: Ten rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at weeks 14, 52 and 104.

Interim Necropsies: Five rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at 4 and 14 weeks for PCNA, palmitoyl CoA oxidase, and compound level samples. (Note samples for

organs weights and histopathology taken at 14 weeks.) Ten rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at 52 weeks for compound levels, organ weight, histopathology samples.

Organ Weights: Measured at 14 and 52 week interim necropsies.

Histopathology: Microscopic examination of selected tissues at 14 and 52 week animals. Complete tissue examination of 0, 2, 5, and 20 ppm 104 weeks animals. Selected tissue examination of 20 ppm recovery animals. Histopathologic results will indicate extend of tissue examination of 0.5 ppm group.

Study Monitor: Andrew Seacat; Alternate, Marv Case

January 21, 1999

Rat Teratology Rangefinder on N-EtFOSE

Study Outline

Study Objective: Explore possible dose levels for an oral rat teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female Charles River CD rats

Dose Groups: Seven - control, 0 mg/kg; low-1, 1 mg/kg; low-2, 5 mg/kg; mid-1, 10 mg/kg; mid-2, 20 mg/kg; high-1, 25 mg/kg; high-2, 35 mg/kg

Number per Group: 8 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 6 thru 17 of gestation. Analysis of dosing preparation will not be done, however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 20 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be sexed, weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

January 21, 1999

Rat Teratology Study on N-EtFOSE

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered N-EtFOSE in pregnant rats

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female Charles River CD rats

Dose Groups: Five - control, 0 mg/kg; low, (1 or 1.5 ?) mg/kg; mid-1, (5 ?) mg/kg; mid-2, (10 or 15 ?) mg/kg; high, (20 or 25 ?) mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 25 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspened in water with 2% Tween 80 on days 6 thru 17 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 20 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be sexed, weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: Approximately one-half of the fetuses from each litter will processed for visceral examination by the Wilson Technique for soft tissue development. The remaining fetuses will be eviscerated and processed for skeletal examination using Alizarin Red S staining method.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (1 or 1.5 mg/kg) and five females at the high dose (20 or 25 mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 18 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

Sponsor Study Monitor: Marvin T. Case, DVM, PhD
3M Toxicology Services
3M Center Bldg 220-2E-02
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Telephone 612/733.5180
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Alternative Study Monitor: Andrew M. Seacat, PhD
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January 21, 1999

Rabbit Teratology Rangefinder on N-EtFOSE

Study Outline

Study Objective: Explore possible dose levels for an oral rabbit teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female New Zealand White rabbits (**Note** the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Seven - control, 0 mg/kg; low-1, 1 mg/kg; low-2, 5 mg/kg; mid-1, 10 mg/kg; mid-2, 25 mg/kg; high-1, 50 mg/kg; high-2, 75 mg/kg

Number per Group: 5 mated females per group

Test Article: N-EtFOSE will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Analysis of dosing preparation will not be done; however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will be examined for the number of corpora lutea.

January 21, 1999

Rabbit Teratology Study on N-EtFOSE

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered N-EtFOSE in pregnant rabbits

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female New Zealand White rabbits (**Note** the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Five - control, 0 mg/kg; low, (1 ?) mg/kg; mid-1, (5 ?) mg/kg; mid-2, (10 ?) mg/kg; high, (25 ?) mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 22 mated females per group

Test Article: N-EtFOSE will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implantations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: A mid-coronal slice will be made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses will be examined in the fresh state using Staples' technique for internal abnormalities. At this time the sex of the each fetus will be determined. After removal of the viscera, the carcasses will processed for skeletal examination.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (1 ? mg/kg) and five females at the high dose (25 ? mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 21 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

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January 21, 1999

Rat Two Generation Reproduction Study of N-EtFOSE

Study Outline

Study Objective: To evaluate the effect of oral administration of N-EtFOSE on the reproductive function of male and female rats (F_0 generation) and on the development and reproductive capacity of the subsequent F_1 generation which were exposed in utero and via lactation.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Five - control, 0 mg/kg; low, 1 mg/kg; mid-1, 5 mg/kg; mid-2, 10 mg/kg; high, 15 mg/kg.

Number per Group: 35 males and 35 females per group

Test Article: N-EtFOSE will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Daily oral intubation of compound suspended in water with 2% Tween 80. Compound administration will start 4 week prior to mating. Compound administration will continue through mating and in the females through gestation and lactation. The F_1 pups will not receive any doses via gastric intubation. Samples of dosing preparations on week 2 and 8 will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: Weekly except when male and females are co-inhabited during mating.

Food Consumption: Weekly except when male and females are co-inhabited during mating.

Estrus Cycles: Daily vaginal smears will be taken from females two weeks prior to mating to determine estrus cycle information.

Mating: After 4 weeks of compound administration, within a dose group one male will be co-inhabited with one female. A female will be determined as mated upon the presence of sperm positive vaginal smear. The day of mating shall be considered as day 0 of gestation.

Early Gestation Evaluation: 10 F_0 females per group will killed on day 10 of gestation. Pregnancy will be determined as well as the number of implanations and the number of early and late resorptions. At necropsy serum and liver samples will be taken from five of the females in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

Male Sacrifice: One week after mating all F_0 male rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The testes, epididymis, prostate and seminal vesicles will be weighed and fixed for possible histologic examination. (Note: testis will be fixed in Bouin's solution; other tissues in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the males in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

Female Parturition: The remaining 25 females will continue until term and be allow to deliver their litters. The offspring (F_1 generation) will be counted, sexed and weighed on postnatal days 0, 4, 7,14 and 21. Offspring will be randomly culled to four males and four females on day 4 postnatal.

Milk Samples: On day 4 postnatal when litters are culled to eight pups, milk curds will be collected from the stomach of the discarded pups from five litters of each dose groups. The milk curds from the pups in a litter are to be combined as a single sample.

Pup Examinations: Pups will be observed daily for moribundity/mortality. Developmental landmarks consisting of eye opening, pinna detachment, surface righting, testes descent, and vaginal opening will be recorded for each litter.

F_1 Dosing: After weaning at day 21 of lactation, the F_1 pups will receive compound by daily oral (gastric intubation) dosing. Dose level on a mg/kg basis will be the same as the pup's dam dose level.

Female Sacrifice: One or two days after weaning of the litter on day 21 of lactation, all F_0 female rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The ovaries will be weighed and fixed for possible histologic examination. (Note: ovaries will be fixed in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the females in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

F1 Pup Neurological Testing: During the 4th week postpartum, the F_1 pups will be evaluated in a passive avoidance test for learning and short term memory retention. During the 10th week postpartum, the F_1 pups will be evaluated in a water-filled maze for neuromuscular coordination, learning, and longer term more complex memory.

F_1 Growth and Reproduction: The F_1 pups will be weaned after 21 days of lactation and litters will be culled to one of each sex. The pups will then allow to grow and body weights and food consumption will be recorded weekly. The F_1 pups will undergo behavioral/functional testing. At sexual maturity, within a dose group, one male will be mated with one female (sibling mating to be avoided). The females will be allowed to litter and raise the F_2 pup through 21 days of lactation.

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January 21, 1999

Rat Two Generation Reproduction Study of PFOS

Study Outline

Study Objective: To evaluate the effect of oral administration of PFOS on the reproductive function of male and female rats (F_0 generation) and on the development and reproductive capacity of the subsequent F_1 generation which were exposed in utero and via lactation.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Five - control, 0 mg/kg; low, 0.1 or 0.2 mg/kg; mid-1, 0.5 or 1.0 mg/kg; mid-2, 2 or 3 mg/kg; high, 5 or 8 mg/kg.

Number per Group: 35 males and 35 females per group

Test Article: PFOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Daily oral intubation of compound suspended in water with 2% Tween 80. Compound administration will start 4 week prior to mating. Compound administration will continue through mating and in the females through gestation and lactation. The F_1 pups will not receive any doses via gastric intubation. Samples of dosing preparations on week 2 and 8 will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: Weekly except when male and females are co-inhabited during mating.

Food Consumption: Weekly except when male and females are co-inhabited during mating.

Estrus Cycles: Daily vaginal smears will be taken from females two weeks prior to mating to determine estrus cycle information.

Mating: After 4 weeks of compound administration, within a dose group one male will be co-inhabited with one female. A female will be determined as mated upon the presence of sperm positive vaginal smear. The day of mating shall be considered as day 0 of gestation.

Early Gestation Evaluation: 10 females per group will killed on day 7 of gestation. Pregnancy will be determined as well as the number of implanations and the number of early and late resorptions.

Male Sacrifice: One week after mating all F_0 male rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The testes, epididymis, prostate and seminal vesicles will be weighed and fixed for possible histologic examination. (Note: testis will be fixed in Bouin's solution; other tissues in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the males in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

Female Parturition: The remaining 25 females will continue until term and be allow to deliver their litters. The offspring (F_1 generation) will be counted, sexed and weighed on postnatal days 0, 4, 7,14 and 21. Offspring will be randomly culled to four males and four females on day 4 postnatal.

Milk Samples: On day 4 postnatal when litters are culled to eight pups, milk curds will be collected from the stomach of the discarded pups from five litters of each dose groups. The milk curds from the pups in a litter are to be combined as a single sample.

Pup Examinations: Pups will be observed daily for moribundity/mortality. Developmental landmarks consisting of eye opening, pinna detachment, surface righting, testes descent, and vaginal opening will be recorded for each litter.

F_1 Dosing: After weaning at day 21 of lactation, the F_1 pups will receive compound by daily oral (gastric intubation) dosing. Dose level on a mg/kg basis will be the same as the pup's dam dose level.

Female Sacrifice: One or two days after weaning of the litter on day 21 of lactation, all F_0 female rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The ovaries will be weighed and fixed for possible histologic examination. (Note: ovaries will be fixed in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the females in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

F1 Pup Neurological Testing: During the 4th week postpartum, the F_1 pups will be evaluated in a passive avoidance test for learning and short term memory retention. During the 10th week postpartum, the F_1 pups will be evaluated in a water-filled maze for neuromuscular coordination, learning, and longer term more complex memory.

F_1 Growth and Reproduction: The F_1 pups will be weaned after 21 days of lactation. The pups will then allow to grow and body weights and food consumption will be recorded weekly. The F_1 pups will undergo behavioral/functional testing. At sexual maturity, within a dose group, one male will be mated with one female (sibling mating to be avoided). The females will be allowed to litter and raise the F_2 pup through 21 days of lactation.

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January 21, 1999

Rabbit Teratology Rangefinder on PFOS

Study Outline

Study Objective: Explore possible dose levels for an oral rabbit teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female New Zealand White rabbits (**Note** the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Seven - control, 0 mg/kg; low-1, 0.1 mg/kg; low-2, 1 mg/kg; mid-1, 2.5 mg/kg; mid-2, 5 mg/kg; high-1, 10 mg/kg; high-2, 20 mg/kg

Number per Group: 5 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Analysis of dosing preparation will not be done; however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

January 21, 1999

Rabbit Teratology Study on PFOS

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered PFOS in pregnant rabbits

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female New Zealand White rabbits (**Note** the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Five - control, 0 mg/kg; low, 0.1 mg/kg; mid-1, 1 mg/kg; mid-2, 5 mg/kg; high, 10 mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 22 mated females per group

Test Article: FOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: A mid-coronal slice will be made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses will be examined in the fresh state using Staples' technique for internal abnormalities. At this time the sex of the each fetus will be determined. After removal of the viscera, the carcasses will processed for skeletal examination.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (0.1 mg/kg) and five females at the high dose 10 mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 21 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

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January 21, 1999

Rabbit Teratology Rangefinder on N-EtFOSE

Study Outline

Study Objective: Explore possible dose levels for an oral rabbit teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female New Zealand White rabbits (**Note** the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Seven - control, 0 mg/kg; low-1, 1 mg/kg; low-2, 5 mg/kg; mid-1, 10 mg/kg; mid-2, 25 mg/kg; high-1, 50 mg/kg; high-2, 75 mg/kg

Number per Group: 5 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Analysis of dosing preparation will not be done; however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

January 21, 1999

Rabbit Teratology Study on N-EtFOSE

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered N-EtPFOS in pregnant rabbits

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female New Zealand White rabbits (**Note** the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Five - control, 0 mg/kg; low, (1 ?) mg/kg; mid-1, (5 ?) mg/kg; mid-2, (10 ?) mg/kg; high, (25 ?) mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 22 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

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Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (1 ? mg/kg) and five females at the high dose (25 ? mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 21 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

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January 21, 1999

Corporate Toxicology Study Outline

Title:

1) Effect of Perfluorochemicals on Bioenergetic Metabolism: Phase II Research Plan (Restricted Grant)
2) Mitochondrial Interactions of Peroxisome Proliferators (Unrestricted Grant)

Purpose:

1) Compare mitochondrial bioenergetics between mitochondria from rat, guinea pig and human (Ca^{++} loading capacity, uncoupling potential, et al.) to help set appropriate safety factors for human risk characterization based on animal data;
2) Compare the metabolic response ([ATP], respiration rate, mitochondrial membrane potential, markers of peroxisome proliferation, et al.) of rat, guinea pig and primate hepatocytes to this class of compounds with particular emphasis on mitochondrial and peroxisomal metabolism to:
   a) Further test the proposed mechanism of toxicity;
   b) Identify potentially useful metabolic biomarkers of exposure;
   c) Help validate relative differences between species (assess markers of peroxisome proliferation across species);
3) Compare molecular response (gene expression → mRNA) of rat, guinea pig and primate hepatocytes exposed to these chemicals (genes indicative of cell and peroxisome proliferation) to:
   a) Identify molecular biomarkers of exposure;
   b) Facilitate species extra[polation;
   c) Discriminate between proliferation of peroxisomes and cell proliferation (ongogenic response) to test dogma;
   d) Assess the relevance of rat tumorigenic response to potential human cancer response;
   e) Provide biomarkers for potential use in monitoring and interpreting traditional toxicity studies.

Significance:

1) Comparing sensitivity between species will allow for judging the most appropriate species for predicting human health outcomes following exposures to compounds and has relevance to establishing safety factors and putting human cancer risk in perspective;
2) Species differences in metabolic and molecular response will reveal important insight into mechanisms responsible for compound-induced peroxisome proliferation and/or tumorigenesis which is also vital to identifying valid biomarkers to assess exposures and potential risks;
3) Comparing the enhanced transcription and expression of genes associated with peroxisome versus cell proliferation will allow opportunities to evaluate the

relationship between metabolic and oncogenic effects of these compounds in different species.

Objective(s):

1. Establish hepatocyte cell cultures from different species as models for assessing the metabolic and mitogenic effects test compounds;
2. Develop molecular probes for assessing compound-induced transcription of genes related to peroxisome metabolism and cell proliferation;
3. Establish benchmarks for assessing the effects of test compounds on hepatocyte bioenergetics and cell proliferation in culture.

Protocol:

1) Establish stable primary hepatocyte cultures from several species including rat, guinea pig and primate;
2) Develop molecular probes for compound-initiated transcription of specific genes related to mechanistic endpoints:
   a) ID genes of interest
   b) ID sequences of high homology between species using Gene Bank®
   c) Design primers to 1.5 – 3.0 kbp sequences (Oligo®)
   d) PCR amplify sequences from rat-liver cDNA template
   e) Separate, bands on low melting point gels
   f) Purify bands (band-stab)
   g) Restriction digest to confirm identity (MacDNAsis® to obtain restriction site map for each probe)
   h) Label probe with ^{32}P-dCTP by random priming (High Prime®)
3) Treat sample (cells or flash frozen tissue)
   a) Isolate total RNA by triazole method
   b) Separate on denaturing HCOH agarose gels
   c) Transfer to nylon
   d) Hybridize with ^{32}P probe
   e) Wash and expose to X-ray film to detect complementary mRNA
4) MRNA probes suggested for peroxisome proliferation
   a) CPT1
   b) ACoAO
   c) PPARα
   d) FABP
   e) Also, possibly Catalase, LPL, Aromatase, HMG-CoA synthetase
5) Probes for cell proliferation
   a) PCNA
   b) CDK
6) Establish benchmarks for assessing the effects of PF compounds on hepatocyte bioenergetics and cell proliferation in culture
   a) Effect of exposing cells in culture on induction of peroxisomal metabolism and stimulation of cell proliferation using biochemical markers and molecular markers (e.g., mitochondroial enzyme activities and cytochrome content, Adenine

nucleotides, AcoAO activity, PCNA, apoptosis (morphometric and TUNEL), CyQuant cell proliferation analysis, CPT1, AcoAO, PCNA, CDK)

Principle investigator/location/cost:
Dr. Kendall Wallace, University of Minnesota School of Medicine, Department of Biochemistry and Molecular Biology, University of Minnesota, Duluth

Timeline:
31 months beginning December 1, 1998 and ending June 30, 2001

Report:
Quarterly progress reports